

BEST AVAILABLE COPY

Tday (see p.29)

Project No. 100.07/000,421

Book No.

65

Date No. —

Trig assay mix (P557)
except no activated DNA

[A]

(40 Rxns)

0.5M Tris pH 8.3	100	✓
1M MgCl ₂	4	✓
3M KCl	33.3 µl	✓
1ATGC-TP (1mM each)	40	✓
2 ³ P dCTP	5.1 4.2 µl	✓
H ₂ O	102.19 µl	✓
	(1) 38 µl → 1.4 ml	(2) (use 3) µl / 5 µl rxn
	385	
5 (same as P17)		← 11 Rxns
3.0mM 1950 pmol/tl/µl	13.3 µl	(40) 11.7 µl
0.165 µg/µl		① = 0.2 µg DNA/5 µl
H ₄ O	96.7	② = 1 µg DNA/5 µl
	45.5	(= 5 nmol/tl/5 µl)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20		
45 µl	87 µl	total units

EKBT1	5	5	5.4
8	5	5	0.8
6	5	5	1.5
25	5	5	3.125
5	5	5	6.25
5	5	5	12.5
5	5	5	25
5	5	5	50
Yml	5	5	100
50 µl	2 min at 74°C → add 10 µl EDTA		200
	spot 40 µl on GFC		

To Page No. —

I read & Understood by me,

Janet Polans

Date

10/24/94

Invented by

Recorded by

Date

10-18-94

Project No. _____

Book No. _____

TITLE _____

on Page No. _____

units / 50 μl2
9
1A1.0
μg
DNA51
②

pmol

0.4	1	133.00	2.6
0.8	2	248.00	4.9
1.6	3	264.00	5.2
3	4	470.00	9.2
6	5	633.00	12.5
12	6	886.00	17
25	7	991.00	19.5
50	8	995.00	19.6
100	9	999.00	19.7
200	10	883.00	17.4
0.4	11	2146.00	42
0.8	12	3847.00	73
1.6	13	6695.00	132
3	14	12077.00	238
6	15	17179.00	339
12	16	17333.00	342
25	17	22279.00	440
50	18	22941.00	452
100	19	23863.00	471
200	20	24510.00	477
	21	92.00	
	22	304197.00	

76 cpm/pmol

need time course at high and low Tag to see if lag plays a role. In a PCR with 15-30 min elongation time, effect of lag would be minimized.

Results $\text{per Tag script} = 10^5 \text{ cpm/mg}$
 $25 \text{ units} = 50 \text{ nM Tag}$
 $5 \mu\text{l}$

Both plots (0.2 and 1.0 μg DNA) saturate a 50 nM Tag suggesting an equilibrium effect of pol DNA binding rather than titration of total pol/Tag. ~~at 10 nM~~
 1 μg DNA, saturation at 10 nM or 42 pmol/cells ~~at 10 nM~~

for 200,000 cpm/Tag

1 unit Tag = 0.53 pmol molecules $\approx 1:1$ Tag/cell

1 μg mp19 \Rightarrow $1 \times 10^{-6} \text{ kg}$
 $(330 \text{ g mole/l}) (7250 \text{ fm}) = 0.42 \text{ pmol/cell}$

Page No. _____

I attest & understand by me,

Dorothy Polcup

Date

10/24/94

Invented by

Recorded by

Date

10-77-94

102

Project No. _____
Book No. _____

TITLE Tag - Mutant \rightarrow Heparin Pool over Super Q G50

From Page No. _____

Heparin Pool \rightarrow dialyzed against Bfr A \rightarrow 250 mL - 2 exch
 $\sim 12 \text{ mL}$

Bump 5 mL Super Q G50 column w/ Gu HCl + NaCl \rightarrow
Wash w/ H₂O
Equilibrate w/ Bfr A inlet conductivity 1.37 mS
outlet conductivity 1.42 mS

Sample - 1.5 mS

Saved 1 mL of Load material - Load $\sim 11.5 \text{ mL}$
Collect Load flow through + wash.

Wash w/ Bfr A - Flow rate - 1 mL/min

Gradient Bfr A \rightarrow Bfr B \rightarrow 10 vts - 50 mL total

Collect 1 mL fractions -

Pool 10-12 dialyze against storage buffer -

Sign premix - add 11 μL hot dCTP -

5 μL / vts - 5, 1, 2, 4 μL - enzyme dilute 1/20

7

D.S	1	62892.00	780
	2	53562.00	
1/20	3	80556.00	Q pool
	4	80834.00	
1/20	5	39642.00	
	6	55734.00	Q pool
1/20	7	61384.00	
	8	69380.00	
1/20	9	49764.00	
	10	42686.00	Hep Pool -
1/20	11	75336.00	Load
	12	60344.00	
1/20	13	50018.00	
	14	57888.00	xp load -
	15	652.00	

1	7280.00	
2	7498.00	12.9 $\mu\text{Ci}/\mu\text{L}$
3	13534.00	
4	2836.00	11.7 $\mu\text{Ci}/\mu\text{L}$
5	4118.00	
6	4440.00	

SA = 78 cpm/pmol

Factor = 1.154×10^{-8}

To Page N

Witnessed & Understood by me,

Date

Waters

Invented by

E. Wagner

Recorded by

Date

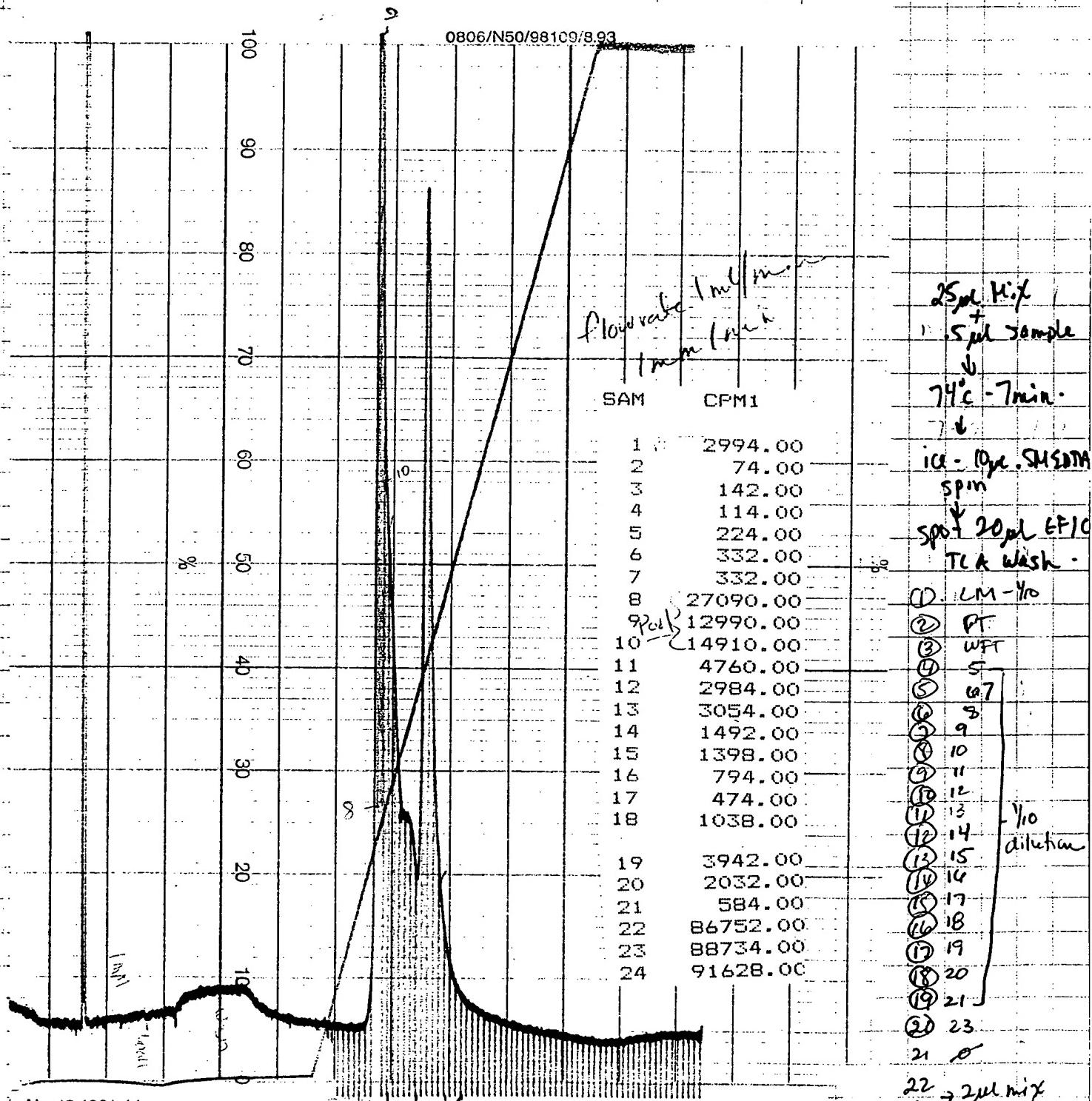
12/94

Super Q-650-

Pr j ct No. _____
Book N . _____

103

age N .



le No. 18-1001-44

To Page No. _____

sed & Understood by me,

Date
2/21/93

Invented by
G. Flynn

Recorded by

Date
1/94

T. neapolitana DLE (2779) 50M

Book No. 3884

sg No. 11

October 13, 1994 (Thursday)

I infected DH12S cells with the ♂ from #5 ♀, 7 (2mL cells grown in 2X YT + 5% v/v ♂; grown 37°C (air shaken) 16 hrs)

RF isolated by alkaline lysis except I've RNase A (1mg/mL) added to prep at NH₄OAc addition

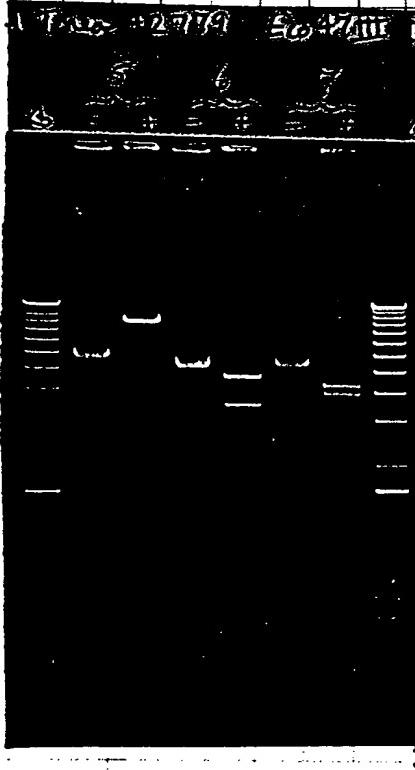
NA dissolved in 50 μL T₁₀E₁

DIGEST SCHEME

HOF	10 μL	↓	Incubated 37°C (heat block) 1 hour.
(React 3) 10X BPK	2		
DNA	7		

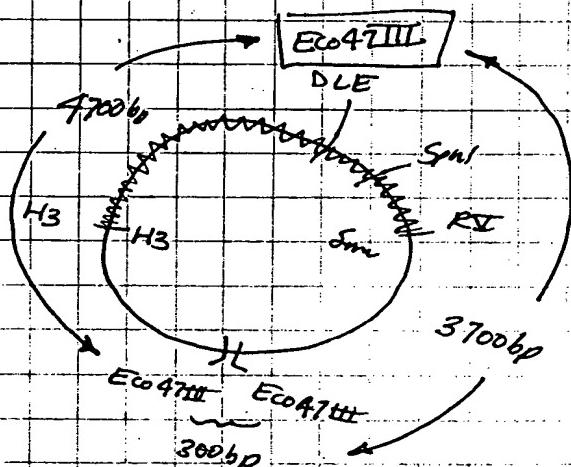
self) Eco 47III	1	↓	Tame 20μL
	+		

3% Agarose Gel (1X TAE); 190V/16W



10/24/94

Comments:



The bands are migrating at the expected distances for #6. There must have been overabundance of some "component" causing the DNA to run faster. I will clone into the pUC vector.

To Page No. 5C

ssed & Understood by me,

Date

Invented by

Date

May Forgo

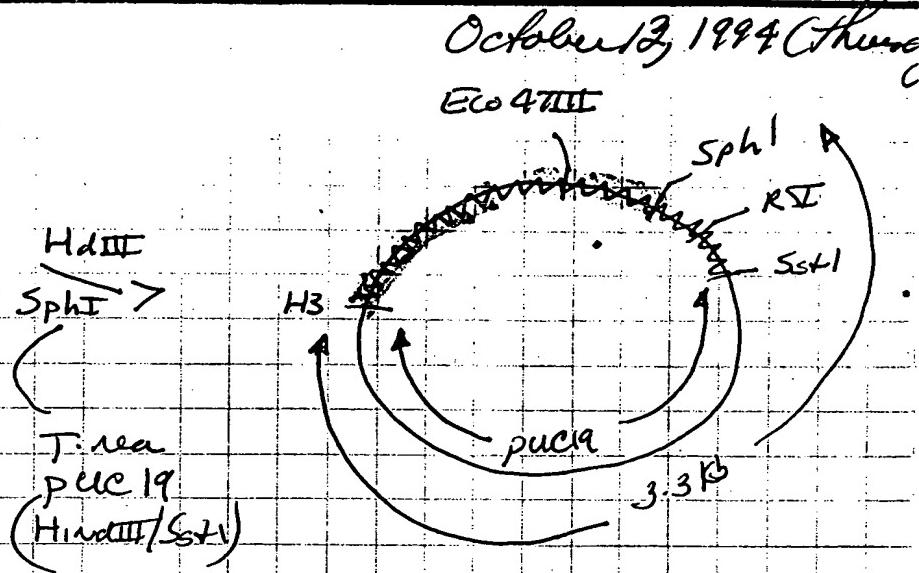
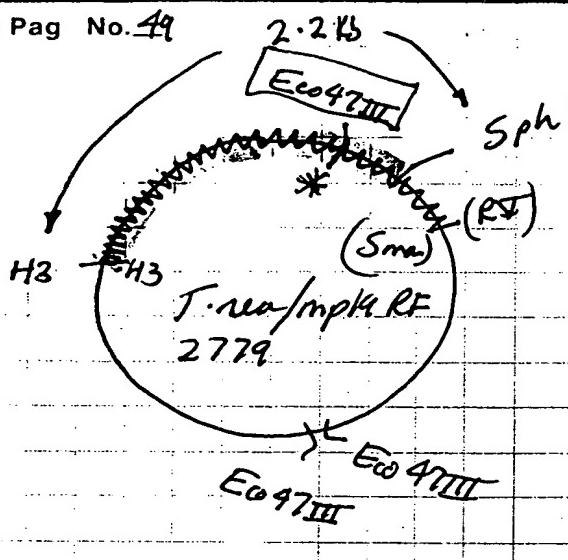
10/24/94

Recorded by

Brian F. Schmidt

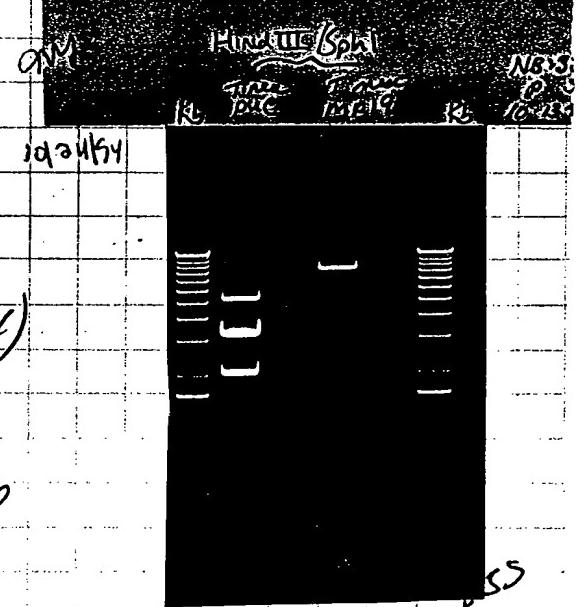
10-13-94

From Pag No. 49

DIGEST SCHEME

	T. nea/pUC	2779/pmp19	#6	
H2O	12 μl	6 μl	✓	Incubated 37°C (short)
(React 2) PXPk	2	2	✓	1:10 → 1.50
DNA	1	12	✓	
(10μg/ml) HindIII	1	1	✓	
(10μg/ml) SphI	1	1	✓	
Torrc	20 μl	20 μl		0.8% Agarose-Gel (XTRA), 196

Comments: I should see a 3.3 kb (desired fragment) and a 2.2 kb fragment from the T. nea/pUC19 clone and I do. Unfortunately I should see a 7.9 kb fragment and 2.2 kb (desired fragment) from the T. nea/pmp19 (2779 #6) RF DNA and I don't. Both sites were present before I performed the mutagenesis (see p. 35) - I will have to repeat.



To Page No

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Date

Invented by

Dat

Mon Jones

10/13/94

Recorded by

Dwight J. Schmidt

10-13-94

16 11/16/94

Project No. _____
Book No. _____

TITLE Deep Vent / GAPDH / dT primers

From Page No. _____

Purpose: Since GAPDH - PCR worked with 3' Thiol primers attempted the same amplification with other available primers, under same conditions.

- Deep Vent buffer 200 µg Terrylite
- dNTP 1 µM
- primers did just one of each: lacZ forward and reverse: lacZ forward and reverse (100 µM)
- * 2697 & 2696
- + dU " "
- each primer set 10x Rx were made.

	Regular	17-20 = 0.5 U	dU	33-36 = 0.5 U	3-1 PPT
10x buffer	50	50	50	50	25
dNTP	10	10	10	10	10
primer 1	5	5	5	20	20
2	5	5	5	20	20
Templati	20 (100 µg/s)		20		20
H ₂ O	860		270		330
	4150	→ 45 µl/Rx ← 450			← 450

added 5µl of Mg chelator	2	3	4	6	mm	67.50
Mg chelator	1	1	1	1		
ml	0	0.5	1	2	(100 mM)	10 x
	5	4.5	4	3	11.20	J

- everyone added individually 0.25 µl for 0.5 U }
- 0.5 µl for 1 U }

To Page No. _____

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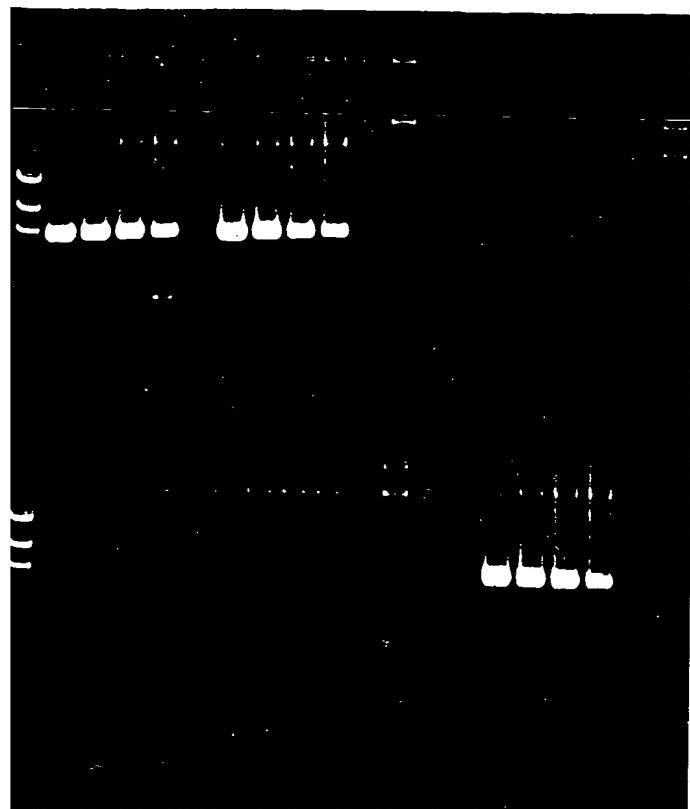
Dr. Schaeffer

11/16/94

g N - Regular

3'-1 PPT

0.5U



il 0.5 2 3 4 5 6 7, 1U, M

dv primer

Vent + 1- exo didn't discriminate between modified and unmodified.

With dv in earlier thesis pcr & 500 bp (ac 2) never got amplified.

- samples dried 12/19/94

Result

- Regular = unmodified, revised, so could only say 3'-1 PPT is better than unmodified!
- dv certainly has problem with Deep Vent.
- with the amount of product seen with 1U don't know why there is no product with 0.5U & 3'-1 PPT primers.

Con of template = ?
 Con of enzyme = ?
 control are there bands on the top.

Template / primer = no enzyme controls also have them?

To Page No. _____

ed & Understood by m .

Date

Invented by

Date

11/18/94

Recorded by

S. Sitaaraman

11/18/94

Clot Assay for T. nec. Pol.

Project No. _____
Book No. _____Exhibit 1
Appl. No. 09/558,421

51

Lab No. 2/1/95 Purpose: DETERMINE TOTAL CONCENTRATION OF TWO SAMPLES.

Premix:

- ✓ 33µl 2x Reaction Buffer (25mM TAPS, pH 9.3/50mM KCl/1mM DTT)
 ✓ 1.3 µl 1M CO₂ @ 1.00M
 ✓ 92.9 µl BSA @ 3.5mg/ml
 ✓ 139.8 180.7 625µl H₂O
 ✓ 65µl H.C. @ 90.3 cpm/pmol

Reaction Buffer:

I. 0.5M TAPS, pH 9.3 100ml
 FW = 243.3 : 12.165g
 TREATED WITH 10N NaOH

II. FOR 10ml @ 2X

- ✓ 1ml 0.5M TAPS, pH 9.3 = 50mM
 ✓ 500 250µl 2M KCl = 100mM
 ✓ 40 20µl 0.5M DTT = 2mM

Assay @ 72°C, 10min

U/ml

	156	17.3
1ul or 53µl @ 100	65413	
2ul	113154	15.0 X = 15.2 U/ml
3ul	151375	13.4
1ul of 5µl @ 100	44554	1.48
3ul	95434	1.58 X = 1.47 U/ml
3ul	121699	1.35
1ul JH-61 Ocar @ 1000	19038	

To Page No. _____

Ass'd & Understood by me,

R. Pless

Date

7/12/95

Invented by

Recorded by

Date

5/23/95

TNE

age No. _____

12/95

Goal: To clone the TNE 35Fy (mut) into pTrcSSA.

WEB	1.0 X R4	30
H ₂ O		5
BspHI F		13
		2
		50 J

pTrcSSA	5
10X R2	2
H ₂ O	"
Neot	1
H3	1

37°C - 1 hr.

Applied 3.0 to 2

0.9% agarose gel

Gel run at 180V

APPLIED TO
60.8% GPE
GEL GEL RUN
AT 180V.

SL. VNOU PRACTICE SET #1
PRACTICE 35 F7 #1
BSP HI

pTrcSSA | 7/14/95

NEOT

MMT

Cut out frag & freeze at -20°C.

pTrcSSA / Neot / H3 cut looks good

CUT OUT 50 bp - 4174

- 5C

4120 bp

pUC TNE 35Fy H# / BspHI gives 1kb, 1.3kb,
+ 8.7 kb frag. Therefore, BspHI cuts
pUC TNE 35Fy H# 3X. There must be
a BspHI in the insert.

13/95

EtOH ppt. Digest.

Dissolved in 20 uL TE

BspHI

5uL
5uL NEOT.

Exo HI

15uL DNA

2uL 10X R2

2uL H₂O

1uL H3 10uL

20uL

Applied to 1 lane
of ± 0.9% agarose
gel. Gel run at 180V

37°C - 1 hr.

To Page No. _____

ssed & Und rstood by me,

Date

Invented by

Dat

J. B. Long

7/13/95

Recorded by
May Long

7/13/95

From Page No. _____

- Take pool from Hep. (cation) and load onto Q (anion) column

BFR A.

(el)

0.5 ml M Tris - 7.4	2.5 ml
0.5 mM EGTA	1 ml
5 mM BME	3.58 ml
10% glycerol	1.00 ml
10 mM KCl	3.3 ml
95-712 dH ₂ O. (mS =	1.9)

- Take 8mls from pool -

is in 5.000 D. dilute 1:5 w/w
500 uL to 3500 uL at
6mls

load at 1ml/min. (1.7 mls)

B = same (+) 1M KCl. (6 times)

Load 8mls into new vials

1. dilute (Hep)

1 - SA	1
2 - Blkg	2
3 - C	3
4 - CTT	4
5 - 10	5
6 - 15	6
7 - 18	7
8 - 20	8
9 - 22	9
10 - 24	10
11 - 24	11
12 - 28	12
13 - 30	13
14 - 32	14
15 - 34	15

61656.00	✓
62.00	
974.00	
76.00	
48.00	
84.00	
3696.00	↑
1112.00	↓

Total min

% Reconcile

Pulse count 3550

Blues pool 1500 - (42%)

Hep count 3550

Hep pool 5470 + (154%)

Q count 1248

Q pool 560 - (45%)

H 16.00 - 1514.00
 H 3.200 - 2178.00
 H 6.400 - 3058.00
 H 16.00 - 2722.00
 H 3.200 - 4326.00
 H 6.400 - 4726.00
 125066.00
 125278.00

Test for Activity on 667 Fy (F667). Needs for fol. activity

post HEP kills

- Done as per pool & protocol.

from Del's clone for 1241, Book 3573, #1 Colen

Colen

> - Spec Act = 78.3

- Looks good! Activity post wh.

Need Del to go over for us To Page N

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

³²P primer for 14¹ Venv
Human spleen DNA

Exhibit 2
Project N _____ Appl. No. 09/558,421
B K N _____

67

lge No. ^{32P} 2633 (to the anchor primer)
follow P. 53 except use more ^{32P} ATP

^{32P} 2633 159 μ M
^{32P} γ ATP 6000 Ci/mmol
10 mCi/ μ l 10-24-94
(1.67 μ M ATP)

5 X kinase buffer
PNK 50 μ l

1' 1 μ l	✓ ✓	(159 pm prime) (2)	\sim 26% primers have ATP is difference in labeling
2.5 μ l	✓ ✓ ✓	dry down	
		(41.8 pm ATP)	1.126 ladder
6.75	✓ ✓ ✓	1 μ l H2O	
0.25 μ l	✓	1 μ l 34Pd CP	
33.75		1 μ l EDTA	

37°C 30 min \rightarrow 5' 55°C \rightarrow add

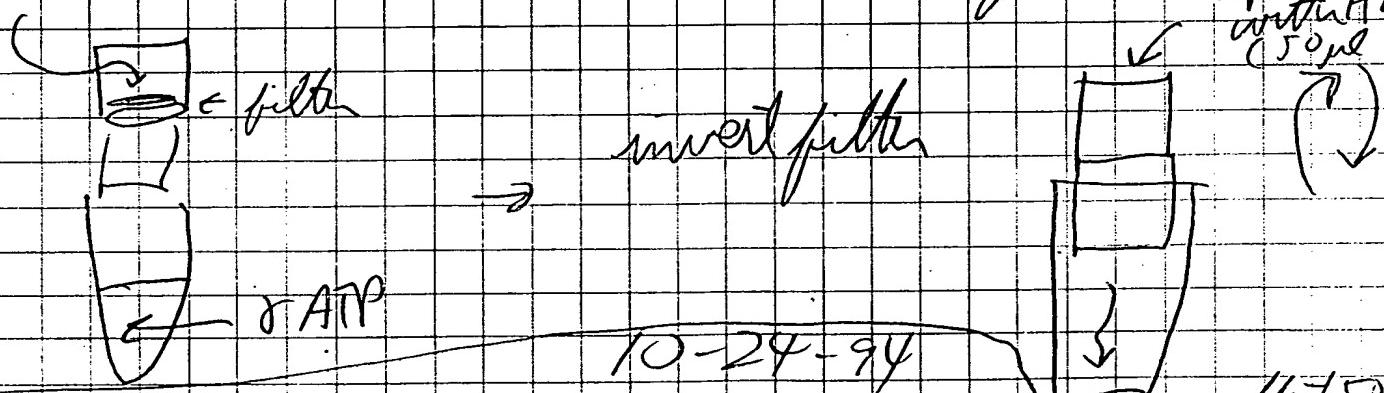
spin col same as P15T, T, and 14S

dilute ^{32P} 2633 with 100 μ l H2O ($V_f = 133$ now)

spin in microfuge in "micron 3"

(anerobic # 42403) - after all venting, put
add \approx 250 μ l more H2O and spin again
remove volume that did not enter filter

spin 5'
without H2O
(50 μ l)



10-24-94

had a problem: filter kept peeling
back on micron 3. Maybe g force was
too high on Beckman microfuge "E" model
will skip separation of free ATP.

^{32P} 2633 is diluted only 33.75 fold for
 $f = 4.71 \mu M$

To Pag No. _____

s d & Understood by me,
Janet Polkay

Date
10/24/94

Inv nted by
Recorded by

Date

10-19-94
10/24/94

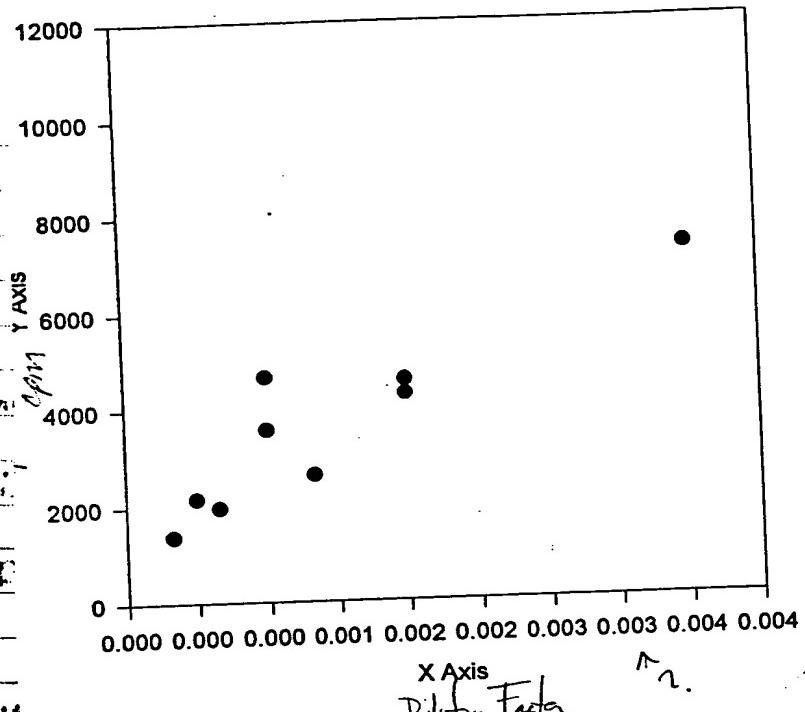
04

Project No. _____

Book No. _____

TITLE _____

From Page No. _____



1	78.00	3.3×10^{-4}
2	1374.00	3.3×10^{-4}
3	1962.00	6.6×10^{-4}
4	2618.00	1.33×10^{-3}
5	2154.00	5×10^{-3}
6	3560.00	1×10^{-2}
7	4262.00	2×10^{-3}
8	4660.00	1×10^{-3}
9	4556.00	2×10^{-3}
10	7268.00	
11	91772.00	
12	92240.00	
13	84328.00	

$\bar{y} = 8.9450$

$$8.9450(25) = 55.9 \text{ cpm/pmol S.A.}$$

$$40,000$$

$$\text{Factor} = 1.61 \times 10^{-5}$$

$$(1.61 \times 10^{-5})(\text{cpm})(DF) - 0/\mu\text{l}$$

48

12

en

heat

V

89.9%

+

V

15

5A

5A

5B

6

30.5

6

34.1

V

45.0

TCI

21.3

27.6

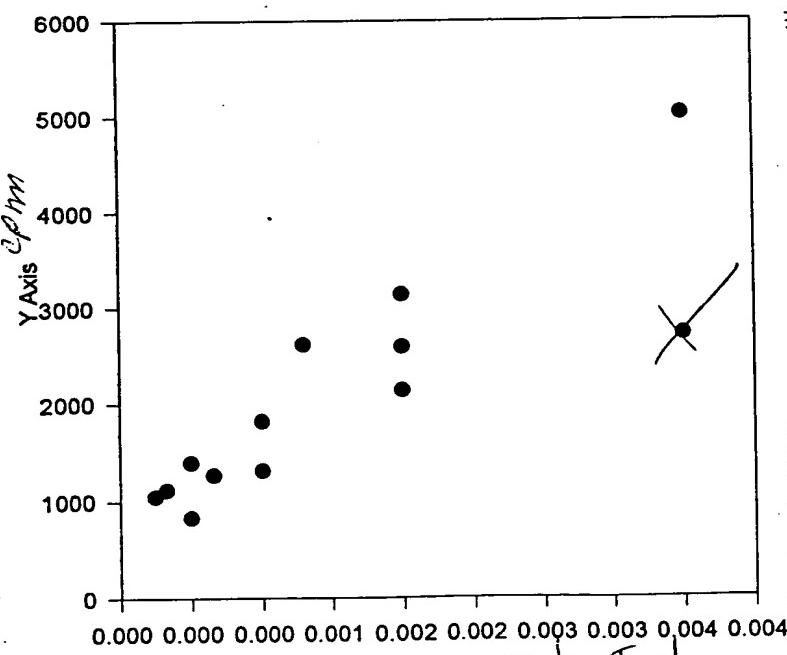
30.7

18.2

22.4

22.5

4x



SAM	CPM1	Date
1	160.00	5/20/94
2	1052.00	5/20/94
3	828.00	5/20/94
4	1116.00	5/20/94
5	1262.00	5/20/94
6	2624.00	5/20/94
7	1392.00	5/20/94
8	1310.00	5/20/94
9	3140.00	5/20/94
10	1820.00	5/20/94
11	2134.00	5/20/94
12	5024.00	5/20/94
13	2592.00	5/20/94
14	2716.00	5/20/94
15	78604.00	5/20/94

$$S.A. \sim 48.7 \text{ cpm/pmol}$$

$$\text{Factor} =$$

To Page N

Witnessed & Understood by me,

[Signature]

Date

5/20/94

Invented by

E. Flynn

Recorded by

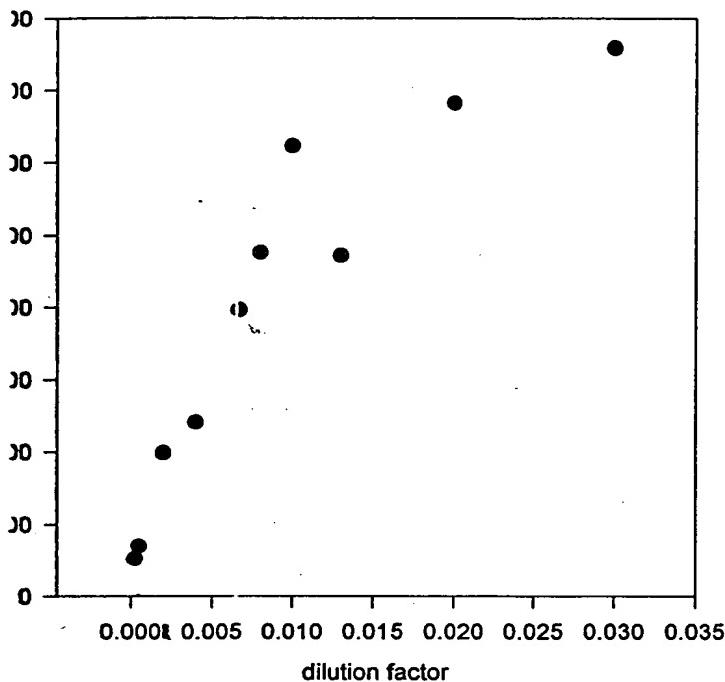
Date

5/20/94

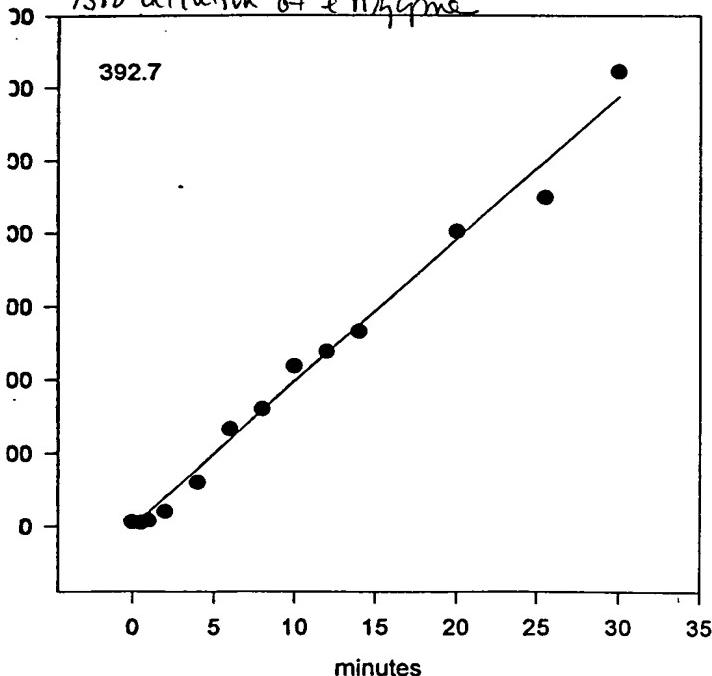
Page N. _____

Mutant Taq Titration

12/13



Time course analysis of mutant Taq - 12/14

 $\frac{1}{50}$ dilution of e. hagyme

		calculated U/ml
1	12470.00	
2	13642.00	
3	15176.00	
4	6342.00	32.9
5	7936.00	20.9
6	9428.00	12.5
7	3970.00	33.0
8	4822.00	20.4
9	9530.00	21.1
10	3978.00	33.0
11	428.00	
12	2624.00	41.1
13	1364.00	
14	578.00	
15	314.00	
16	77492.00	
17	76814.00	
18	79502.00	

$$\bar{x} = 77936.4 \pm 10\%$$

pm

 ~ 25 U/ml

$$\text{Factor} = 1.85 \times 10^{-5}$$

Keep mix - Add 10 μ l .5M
EDTA to terminal
tubes

SDS, μ l premix
1 μ l dCTP 20

10 μ l K2O Tag-1

heat \downarrow 74°C

take out 25 μ l
 aliquots at set
intervals into
separation
tubes -

Spot 20 μ l
GFC filters

TCA wash

SAM	CPM1
1	100.00
2	94.00
3	160.00
4	396.00
5	1198.00
6	2630.00
7	3170.00
8	4340.00
9	4748.00
10	5322.00
11	8060.00
12	9000.00
13	12464.00

24 μ l per mix 2 per run

5 μ l eggysress time point

\downarrow 74°C

25 μ l aliquots • 10 μ l EDTA - spot 20

To Page No. _____

Signed & Understood by me,

HJ

Date

12/14/94

Invented by

E. Hagyme

Date

12/14/94

Recorded by

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

SAM CPM1 μ /ul

1	15576.00	54.5
2	150027100.00	35.1
3	48258.00	50.1
4	12950.00	57.3
5	10003538.00	34.5
6	42578.00	
7	84.00	
8	71702.00	
9	70582.00	
10	67698.00	

 $500 \mu\text{l} \text{ water} + 1.1 \mu\text{l} \text{ ac def Pd}^{32}\text{P}$ ↓
48 μl mix+ 1.2 or 4 μl enzyme↓
10 min. @ 24°C↓
quench w/ 10 μl .5M EDTA + ice↓
spot 20 μl on GF/C - TCA wash

SA. - 43.7 cpm/pmol

$$\text{factor} = 2.0 \times 10^{-5}$$

$$(\text{cpm})(\text{factn})(\text{DF}) = \text{U}/\text{ul}$$

To Page No. _____

Witnessed & Understood by me,

✓

Date 2/2/94

Invented by S. Hansen

Recorded by

Date

12/94

T. neapolitana SDMProject No. _____
Book N. _____

January 25, 1995 (Wednesday)

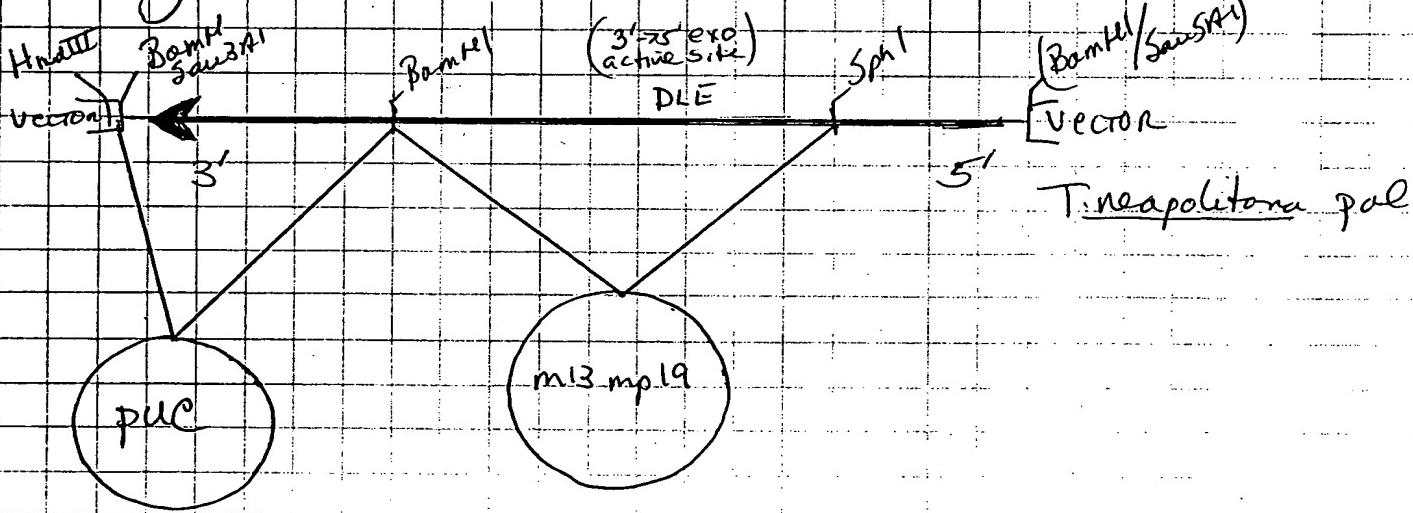
ge N. _____

I'm BACK!!

after a four of duty with Joel's group, a trip to Amherst and a few Greek vacation off I am back and ready to administer the lab below to this project. I will finish sequencing this gene, my luggage is so conform to our needs, and there is so many people can enough enzyme to swim in it and still have money left over for a cup of coffee and a copy of the New York Times!

Now that's for an opening!

First things first. Let's reclone the region of the pol gene we are interested in mutagenizing. Rab and I have had no success with the last clone. Secondly, let's make the subclone more user friendly by eliminating the 3' BamHI site.



stry BsmBI/SmaI

Pcr

Clone HincII/BpuMI

make ssDNA
D → A by SDM

To Page No. 52

ed & Understood by me,

Date 1/27/95

Invented by

Date

Ray Longo

4/24/95

Recorded by

1-25-95

From Page No. 51

January 25, 1995 (Wednesday)

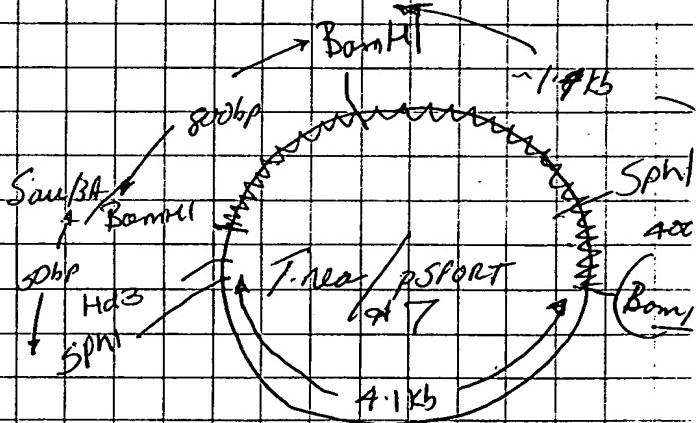
*T. neapolitana / pSPORT DNA made by Michael Smith
(not the Nobel Laureate; the Horse boy)*

DIGEST SCHEME

	<u>T. nea / pSPORT</u>	<u>mBap 19</u>	<u>pUC 18</u>
(vector)	10 μl	13 μl	13 μl
10x BPR	2	2	2
DNA	1	1	1
(ouf/uf) BamH1	1	1	1
(ouf/uf) Sph1	1	1	1
Torsz	20 μl	20 μl	20 μl
(0.1 μg) CAP			1 μl

Incubated 37°C (heat-block) 1:00 → 2:45

0.8% Agarose Gel (1X TAE)
190 Vols



I forgot to run the 1Kb ladder.
What a horse boy!

Fragment T. nea / pSPORT? BamH1/Sph1 show
the 4.5 Kb, 1.4 Kb, 0.8 Kb, 0.05 Kb.
Perhaps something partialled. Try again
but do it separately.

To Page N

Witnessed & Understood by me,

Date

Invented by

Date

Mark Jones

1/27/95

Recorded by J. Schmitz

1-25-95

100

Project No. _____
Book No. _____

TITLE F6674-

From Page No. _____

Span Down 1.2L cells - 0.5-5 - 7000 RPM - 30 min - Decant /
- Dissolve 8.6 grams cells in 25 mL Cold BFR → (Pg 7)
centrifuge 4 X 3.5 30 sec. (t) & at 45 30 sec each

A 5%o 1.200 dia

Crude - .98 ≈ 70% crude. → Heat 15 min @ 88°C.
Final - .13 cool 10 min / ice

ADD 0.4% PEG (1.2 mL 10% stock) - STIR 15 min

Span in 55-34 9K - 30 min - Decant 5%
7.5 min → ADD NH₄SO₄ - 305g/l (1/8 i. cut) - STIR 4°C 45 min
- Span down in 55-34 12 to 12K - Decant 5%, save for next day
Resuspend pellet in 8 mL 3% PEG - Dialyze 48h @ 4°C 15 500mL

BFR. A

25 mM Tris-7.4f

8% glycerol

0.5 mM EDTA

10 mM NaCl

5 mM BME (1.3mL)

BFR. B (10% salt gradient)

Same (1) 2M NaCl

TDSO-650 Hepes

Down Surl column Equilibrate w/ A ✓

wash at 0.5mL/min

wash w/ 8 vts - or till baseline at 1.5mL/min

* Change dialysis buffer once -

Bump 4mL TDSO 650 Apatin - 4M Gu HCl - 3M NaCl 10vts
wash with H₂O -

Equilibrate w/ BFR A - C_{WAD} = (1.4mL) WAD = 7.1mL -
WAD = 2.75 mL/min.

400

10 vts - gradient t → 15 - some protein

Mix - premade by H.G. - stored @ 420°C - same mix as fr nature

With ss d & Understood by me,

Date

2/2/05

Inv nted by

Dat

12-520f

R c rd d by

Page No. _____

purpose: Amplification of pMC9 with different amounts of Tag - dilution.

Dependent

Tag + Dependent

all samples discarded

Constant

different con - dilution

using DV primers, 1 μM

200 μM dNTP

1 μM primer dv x 2728 + 2729 (100 μM)

100 μg template diluted (100 ng) → 1 ng/ μ

Dependent buffer

Dilutions: a. Tag: 0, 0.5, 1, 1.5, 2, 2.5 and 5 μ b. Tag: Deep Vent: 1:0, 1:0.001, 1:0.005, 1:0.01, 1:0.05
1:0.1, 1:0.2, 1:0.5, 1:1, 1:2

c. Deep Vent: 0, 0.025, 0.05, 0.1, 0.5, 1, 2, 5

prepared a master mix w/o enzyme later added separately

Did just one of each.

11/18 1167

10 x buffer 150

primer 15

2 15

Tag: 5 μ diluted to 1 μ / μ in 1x buffer. Template 3 μlEnzyme H₂O

0	=	-	5	D.V	20/ μ → 10/ μ → 0.10/ μ 45 μl	0.0010/ μ
0.5	=	0.5	4.5			
1	=	1	4	0.025	2.5	0.01
1.5	=	1.5	3.5	0.05	5	
2	=	2	3	0.1	1	0.1
2.5	=	2.5	2.5	0.5	5	
5	=	5.0	4	1.0	0.5	2.0
				2.0	4	5.0 = 2.5 μl
						To Page No.

8 - 15

Signed & Understood by me,

Date

11/18/94

Invited by

Date

Recorded by

11/18/94

V. Srinivasan

From Pag No.

T L E D.V

Tag: 14/x

D. ✓

chuklid in

Tirke 8

1 x buffer

Y-axis (microns)	X-axis (number of particles/ml)
0	1
0.001	10
0.005	5
0.01	10
0.05	50
0.1	100
0.5	50
1.0	100
2.0	100

Thermocycled at 94°, 5'

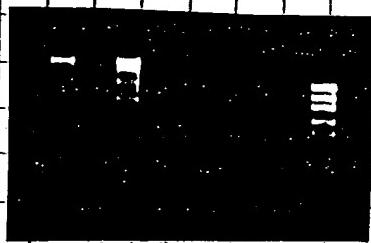
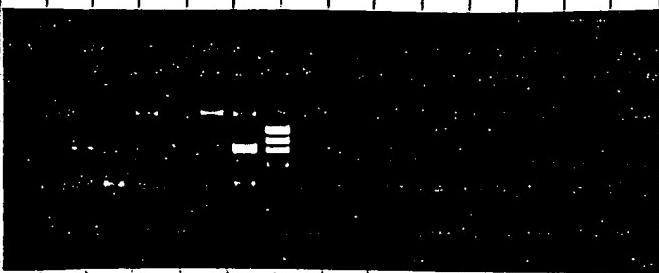
$$30 \left(94^\circ, 30', 56', 45 \text{ sec} \right) 72^\circ, 3')$$

- after 6 cycles noticed dNTP was not added (1) so added 1μl of 10mM dNTP (200 μM / 50 μl x) individually each time & started again the cycling!

may be have to repeat again.

Jag alone Deep vent' alone

Tag : Deep vent



No-contamination? X
(w everyone)

100 100 100 100 100

- so much misprision

Report as in dyspepsia, male Lambde / 17 indig mother

To Page

Project No. _____

Book No. _____

157

Date No. _____

12/95

Goal: To clone the TNE 35F Y (mut) into phage A.

PUC TNE 35F Y Clone H 1	30	pBRSSA	5
BB 1.0XR4	5	10XR2	2
H ₂ O	13	H1.0	11
BspHI F	2	NcoI	1
	50 J	H3	1

37°C - 1 hr.
Applied 3:1 to -
0.9% agarose gel
Gel run at 180V
Applied to 6.0.5% agar
gel Gel run at 180V.



APR
cut out frag & freeze at -20°C.
pBRSSA / NcoI / H3 cut looks good
cut out 56 bp - 4174
- 56 4120 bp

PUC TNE 35F Y H \$ / BspHI gives 1kb, 1.3kb,
+ 2.7 kb frag. Therefore, BspHI cuts
PUC TNE 35F Y #1 3X. There must be
a BspHI in the insert.

13/95

EtOH ppt. Digest.

Dissolved in 20 uL TE

BspHI

Exo HI

5uL

5uL 1XBT

10 uL

15uL DNA

2uL 1XR2

2uL H₂O

1-1 H3 1:0.1

20 uL

37°C - 1 hr.

To Page No. _____

ssed & Und rstood by me,

Date

Invented by

Date

L. B. X.

7/14/95

Recorded by
May Long

7/13/95

^{32}P primer for 14 k Venv
Human spleen DNA

age N .	^{32}P 2633	(its the anchor primer)	$n^{32}\text{P}$ primers have ATP in "so so so effective" in labeling
	follow P. 53 except use more ^{32}P ATP		
oligo 2633	1.59 μM	1 μl	dry down
^{32}P γ ATP	600 Ci/mmol	2.5 μl	1126 ladder
10 $\mu\text{Ci}/\mu\text{l}$	10-21-94		(41.8 μm ATP)
(1.67 μm ATP)			1 μl H ₂ O
5 \times kinase buffer		6.75	1 μl ^{32}P CP
PNK 50 $\mu\text{l}/\text{ml}$		0.25 μl	1 μl 37°C
		33.75	1 μl EDTA

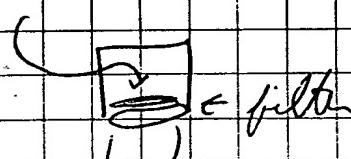
37°C 30 min \rightarrow 5' 55°C \rightarrow add

spin col same as P154,7, and 145,3

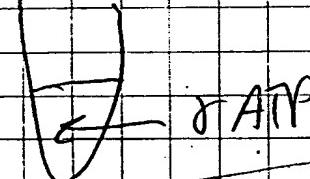
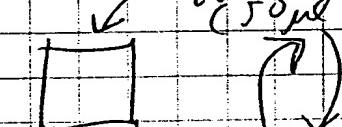
dilute ^{32}P 2633 with 100 μl H₂O ($V_f = 133$ now)

spin in microfuge in "micron 3"
(Beckman # 42403) - after all went up, put
add \approx 250 μl more H₂O and spin again
remove volume that did not enter filter

spin 5'
with H₂
50 ml



invert filter



10-24-94

collect 50,
- it has
oligo

Had a problem: filter kept peeling
off on micron 3. Maybe g force was
too high on Beckman microfuge "E" model
will repeat separation of free ATP.

^{32}P 2633 is diluted only 33.75 fold for
 $C_f = 4.71 \mu\text{M}$

To Pag No.

ssed & Understood by me,

Susan Polkay

Date

10/24/94

Invent d by

Recorded by

Date

10-19-94
10/24/94

Project No. _____

Book No. _____

TITLE 100gram Crack Tne.

From Pag No. _____

Growth: 764 D1 00 IR - 100g

Slurry cells in 200 ml of crack buffer - 1g : 2ml
Final Volume - 300 ml -

Crack buffer -
25mM Tris pH 7.4
1mM EDTA
1mM PMSF
8% glycerol
5mM Bme

Filter cells slurry through 4 layers
of cheese cloth
Pass through gauze (min) 2x Ø 100

Set up 90°C heat bath before cracking cells. Use -
floor shaker -

Incubate Ø 85°C for 12 minutes - with light shaking -
cool immediately on ice water bath ~ 15 min -

Spin Ø 18,000 x g in 6SA rotar - collect supernatant -
- 40 min - bright yellow color -

DE 1 precipitation - 4.1. DE 1 + 50 mM KCl final concentration
add slowly over 30 minutes
ut stir an additional 45 min - spin down Ø 18,000 x g
in 6SA rotar - 40 minutes -

MS $(\text{NH}_4)_2\text{SO}_4$ precipitation -

Add $(\text{NH}_4)_2\text{SO}_4$ solid to a final of 60% saturation -
Ø Add slowly over 30 minutes - ut stir o/N Ø 4°C

100ml 250

To Pag N

Witnessed & Understood by me,

Date

Invented by

E. Flynn

Dat

03/29/95

Rec rd d by

May Long

4/5/95

T. neapolitana 50M

tag No. 52

January 26, 1995 (Thursday)

DIGEST SCHEME:

	Treat/psort	m13 mp 19 (~2700bp)
H2O	24.5 μl	✓
I (heat 3) 10Xβ P/S	3	✓
DNA	1	✓
F107 (10μg/u) BamH1	1.5	✓
TDM	30 μl	30 μl

Incubated 37°C (heat-block) 8:04 → 9:08

✓ 3 μl removed for analytical gel.

	Treat/psort	mp19	
DIGEST	27 μl	✓	
IMKCE	2	✓	
H2O	9	✓	
BamH1	2	✓	
TDM	40 μl		

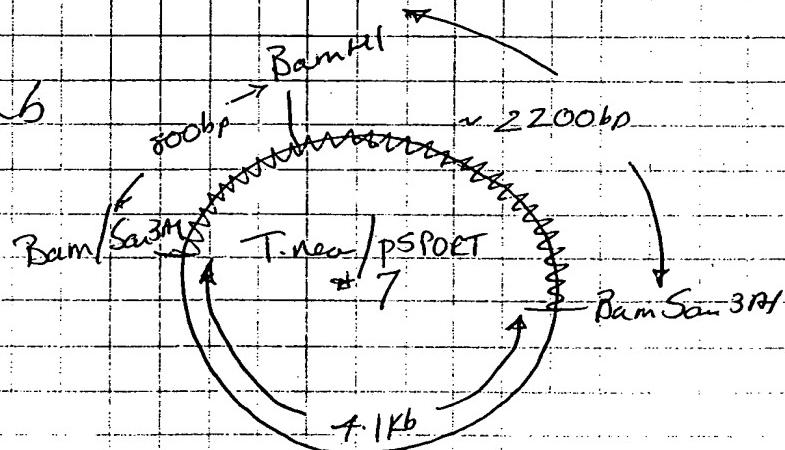
} Incubated 37°C (heat-block)
9:17 → 10:25

Agarose Gel (1X TAE)
190 Vol b.

1/2/95

PJS

Comments



To Page No. _____

Ised & Understood by me,

Date

Invented by

Date

May Tongo

1/27/95

Recorded by

Dr. J. Schmidt

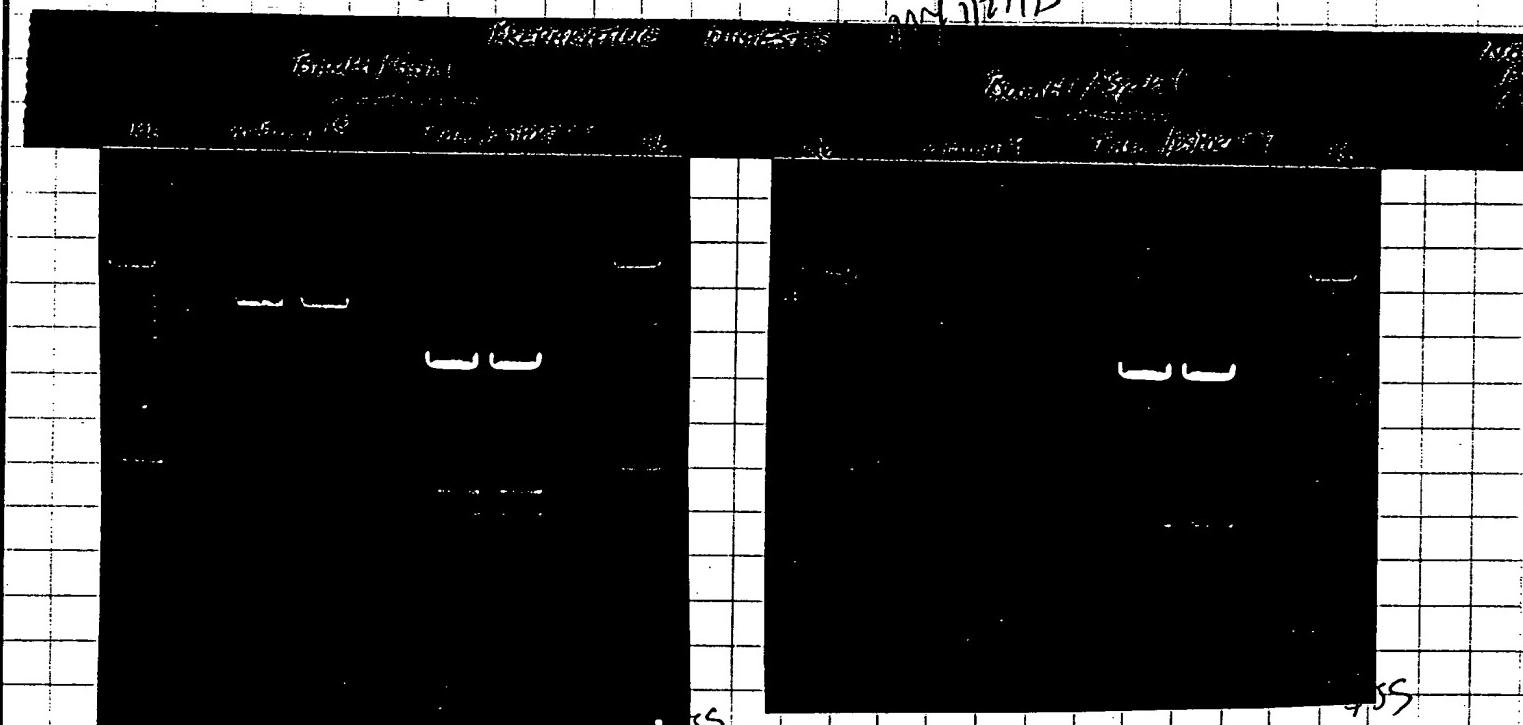
1-26-95

From Page No. 53

January 26, 1995 (Th)

0.8% Agarose Gel (1X TAE); Run at 190 Volts

1/27/95



Bands extracted from the gel and placed in the same tube. The DNA was purified away from the agarose using Gene Clear as described by the manufacturer (BIO-101).

DNA eluted in 14 ml 10H

LIGATION SCHEME

EL. 202 (Ligase) 5X BHK	DNA 14 ml	✓	
	4 ↑	✓	
14 μl (Ligase) form	2 ↓	✓	

Inculated 22°C (room-temp)
2:15 → 3:15

→ 1 μl ligation / 2 μl
for transformation

T Page

Witnessed & Understood by me,

Date

Invent'd by

Dat

Mary Lamp

1/27/95

Recorded by

J. Schmid

1-26-95

T. neapolitana 50N

Pr j ct No.

B ok No.

55

ag N 54

January 26, 1985 (Thursday)

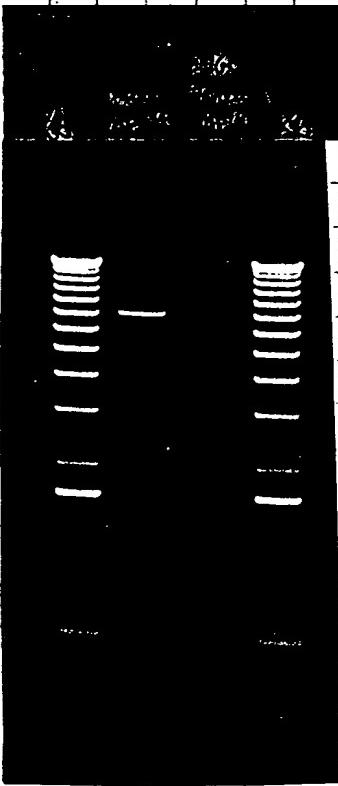
DH10B Cleopatra

20 µl DH10B Competent Cells + 1 µl (of a 1/3 dilution; See p 54)
2.5 KV

1 ml LB, 37°C on shaker 20 min

↳ 10% applied to LB plate in Am 1 Soft Agar (67)
90% + IPTG (1mM) and X-gal 100µg of 4%

incubated 37°C overnight



MM

1/27/95

To Page No.

ssed & Und rstood by m ,

Dat

Invented by

Date

May Longo

1/27/95

Rec d by

David J. Schmidt

1-26-95

Page No. _____

Process: pMC9 amplification, using dU primers # 2722 & 2729

aq titration: buffers / KlenTaq
Template

cycling:

10 μM dNTP

μM primer each

2 μl template pMC9/βgal II

0.2M Mg

94° 3'

80 (94, 30, 56, 30, 72, 3)

30° 1'

56° 1'

30° 1'

72° 3'

prepared 15 x 4/10 enzyme → added separately in 1x buffer

x buffer (D.V.)	75	(K7)	75	5	50/λ	1 ml
1N.T.P.	15		15	2.15		.5
primer 1	7.5		7.5	2		2
2	7.5		7.5	1.5		1.5
Mg	—		15.0	1		1
complexe	3.0		3.0	.5		.5
H ₂ O	642.0		627.0	0		0

distribuid 50 μl / tube added enzyme.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
		16	17										
		18	19										
		20	21										
		22	23										
		24	25										
		26	27										
		28	29										
0.01	15												
1		30											
deepwell mix		7											

↑

rep Vent buffer KlenTaq buffer

T Page No. _____

is d & Underst od by m ,

Dat

11/21/94

Invented by

Record d by

R. Abramson

Date

11/21/94

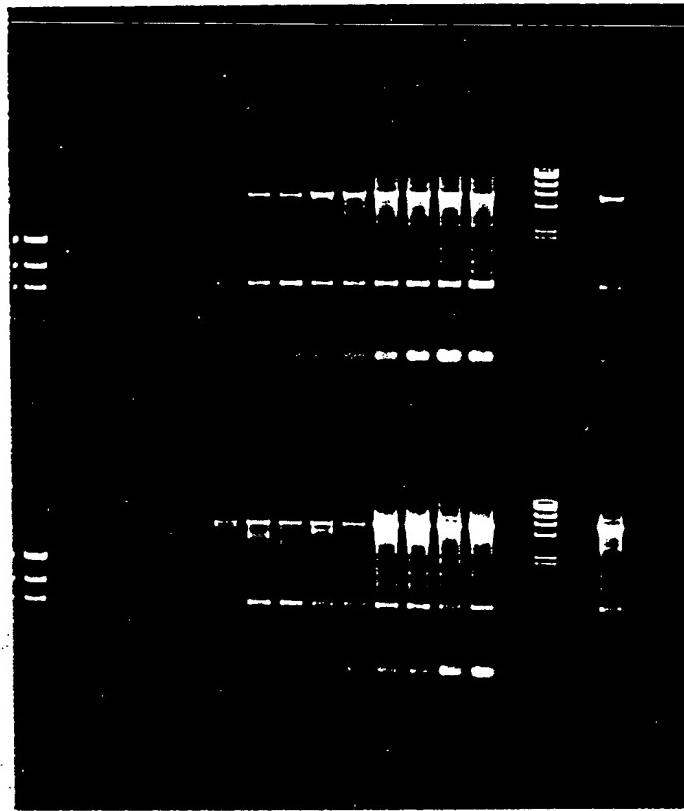
Proj ct No. _____

Book No. _____

TITLE _____

From Pag No. _____

0 . 5 1 1.5 2 2.5 5

0 . 5 1 1.5 2 5
2.5 1:0.01 mixTag liberation

← D.V. buffer

← K.T. buffer

Result: more product with increasing amounts of Tag expected.

- K.T. B / 1U better than D.V. / 1U
- 1: 0.01 lot better than 1 U Tag alone.
- K.T. B more product than D.V. buffer
- But lot of misrunning - adjust the cycling conditions

T Pag 1

Witnessed & Understood by me,

Date

Invented by

Record d by

Dat

11/22/94

TNE

Project No. _____
Book No. _____

159

Page N. _____

1/95

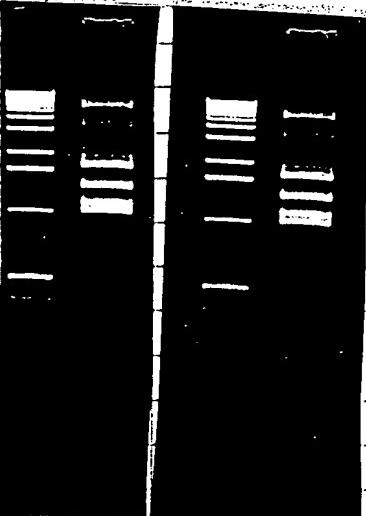
put TNE 35% / BspH I ETOH ppt. → Dissolved in 200ul 1X.

7/17/95 buffer 2.0 l of H3 (100l:1) was added. 37°C - 1h.

applied to 1 lane of a 1% LMP agarose gel.

Cool from at 180V.

1/18/95 put TNE 35% / BspH I H3



✓
7/20/95

cut the 200bp frags out &
freeze at -20°C.

200bp frag

7/17/95

~~GS~~ Used the ^{over} phenol extraction method to purify RNA.
Dissolved in 100l TE.

To Page No. _____

ssed & Understood by me,

Lizu Xu

Date

7/20/95

Invented by

Recorded by

Mary Longo

Date

7/18/95

TOSO - Hg 650(m)

Proj. ct No. _____
Bo k N . _____

ag N . _____

0806/N5C/981C9/8.93

100
90
80
70
60
50
40
30
20
10

1 99310.000
2 594.001PT
3 376.00WPT
4 732.005
5 412.001D
6 642.0012
7 412.0014
8 3694.0046
9 40698.0019
10 75282.0020
11 93378.0022
12 54712.0041
13 23200.0026
14 12038.0028
15 13462.0030
16 1270.00
17 94756.00

74°C-8inch

24hr Run wix
1st cycle - STOP 11
1st cycle - STOP 20 min
X-Cut - 50% FC

1 L 5
2 LPT
3 WPT
4 5
5 10
6 12
7 14
8 16
9 18
10 20
11 22
12 24
13 26
14 28
15 30
16 36
17 54

Holdings

Pool - 1777

Dialy feed
BFR. 4.1-

Code No. 18-1001-44

To Page No. _____

Read & Understood by me,

[Signature]

Date

2/17/95

Invented by

[Signature]

Date

2/18/95

Human spleen DNA

Proj ct N _____
B k No. _____

age N ^{32}P 2633 (to the anchor primer)
 follow P. 53 except use more ^{32}P ATP

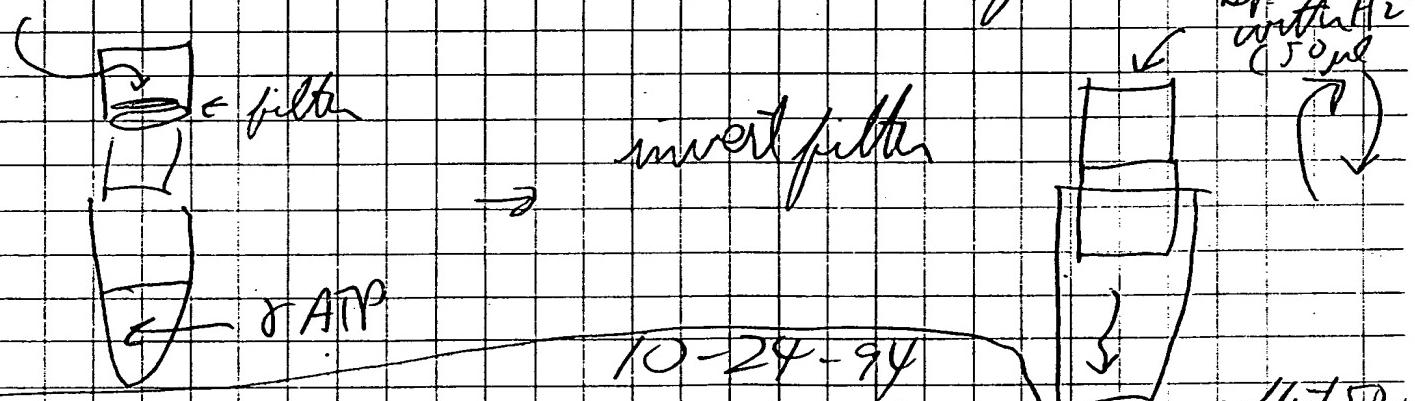
≈ 26 % primers have ATP in
 ≈ 50 % primers effective
 in labeling

iso 2633 159 μM	1 ml	✓ ✓ ✓	dry down
^{32}P γ ATP 600 Ci/mmol	25 μl	✓ ✓ ✓	1126 ladder
10 $\mu\text{Ci}/\mu\text{l}$ 10-24-94			(41.8 pm ATP)
(1.67 μmM ATP)			1 μl H ₂ O
5 \times kinase buffer	6.75	✓ ✓ ✓	1 μl 34Pd (SP)
PNK 50 μl	0.25 μl	✓	15' 37°C
	33.75		1 μl EDTA

37°C 30 min \rightarrow 5' 55°C - add

spin col same as P, 5+, 7, and 145-3

dilute ^{32}P 2633 with 100 μl H₂O ($V_f = 133$ now)
 spin in microfuge in "micron 3"
 (anicon # 42402) - after all went up, put
 add ~~200~~ 200 μl more H₂O and spin again
 remove volume that did not enter filter



Had a problem: filter kept peeling
 off on micron 3. Maybe g force was
 too high on Beckman microfuge "E" model
 will repeat separation of free ATP.

^{32}P 2633 is diluted only 33.75 fold for
 $\text{f} = 4.71 \mu\text{M}$

To Pag No.

s d & Und rstood by me, <i>John Doherty</i>	Date 10/24/94	Invented by <i>J.D.</i>	Date 10-19-94
		Recorded by	10/24/94

Project No. _____
Book No. _____TITLE 13.5 Kb long PCR

From Page No. _____

32P 2633 4.71 μM

✓ 4.7 μl

4.7 μl

0.2 μl

2628 old 199

dilute to 10 μM

✓ 2.2 μl →

0.2 μl

80 ng/μl Human
spleen DNA

✓ 1.1 μl →

(80 ng/)

4 μM NTPs 10 mM each

✓ 2.2 μl →

20 μM

Pol Max

Vent 2 μl

0.5 μl

T7P1 1 μl

15 μl

Vf = 15.5 μl →

✓ 1.36 μl

4.08

Total
(1.32 unit
0.08 μl Ven
in (1). 35X buffer
Cheng

✓ 2.2 →

more in (2) =
0.28 μl VentMg (OAc)₂
12 mM

✓ 1.1 →

C = 1.2

H₂O

32.2/100

65.44

62.7

75.34

72.6

Up = 110 μl

110 μl

remove 10 μl to 2 μl 0.2 M EDTA at 0 cycles.
remove 10 μl at 5, 10, 15, 20, 25, 30, 36

Program 139

15", 94°C → 20 min, 68°C

140

10', 72°C

Started at 8:16 AM
20.5 min/cycle

141

1', 94°C

need 12 hr, 20 min to
complete so expect to end
at 8:40 - 9:00 PM

142 = 141, 139, 140, 4

T Pag N

Witnessed & Understood by me,

Deborah Polans

Date

10/24/94

Invent'd by

Record'd by

Date

10-24-94

Proj ct No._____

Book No._____

69

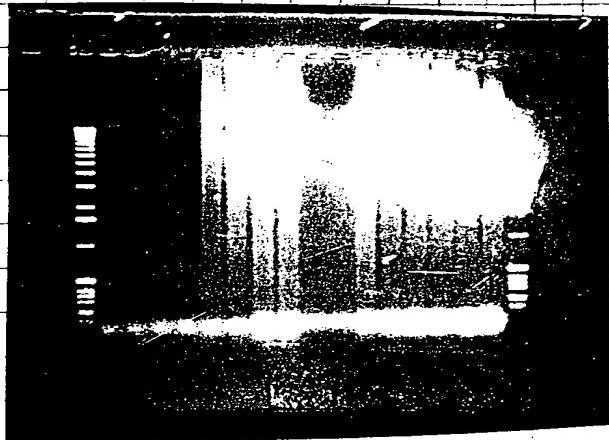
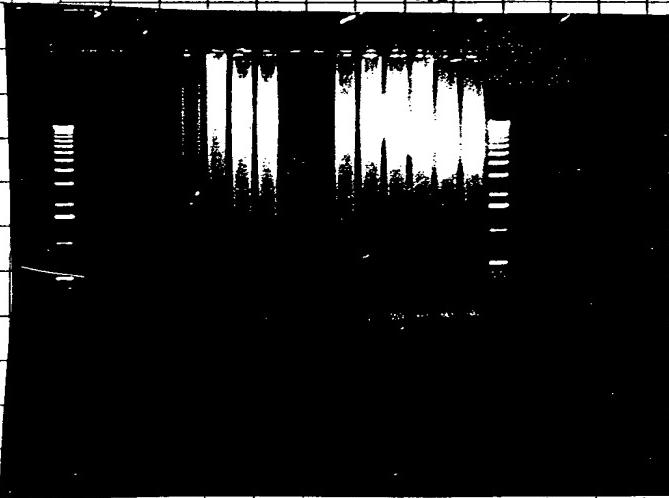
ig N. _____

8% agree same as P.56

Tf) :

1.33 4

05 10 11 2021 3036 05 10 15 2021 7056



To Pag No._____

ed & Understood by me,

ever Bolens

Date

10/24/94

Invent d by

Recorded by

Dat

The - over Heparin 40mL column.Pr j ct No. _____
B k No. _____

Tag No. _____

DD in $(\text{NH}_4)_2\text{SO}_4$ so' n - (B) $18,000 \times g$ 40 minutes -
in GSA rotat -

Save supernatant -

Save pellets -

Save one pellet in - -20°C - process the other
pellet 2

2: pellet 1 slightly greater than half - ~ 3/5

pellet 2 slightly less than half - ~ 2/5

resuspend pellet in 20 mL of Buffer 1 -

Buffer 1

5 mM Tris pH 7.5

dialyze - against Buffer 1 for ~8hrs -

3.1 glycerol

40 mM KCl

Exchange buffer 4 times -

5 mM Bme

.1 mM RNase

parin column - use prepacked Heparin from AG -
n 40 mL column - bump up to HCl + KCl -
wash w/ H₂O -Previously 1.6 used 40 3mL Heparin a
5 gram crakestreet scale up = $\frac{3}{5} = \frac{Y}{~50\text{gr}} = 30\text{mL Heparin}$ equilibrate w/ Buffer 1 \Rightarrow (Note: made 20mM KCl -)

To Page No. _____

Used & Understood by me,

Date

Invented by

Date

May 1995

4/15/95

Recorded by

08/30/95

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

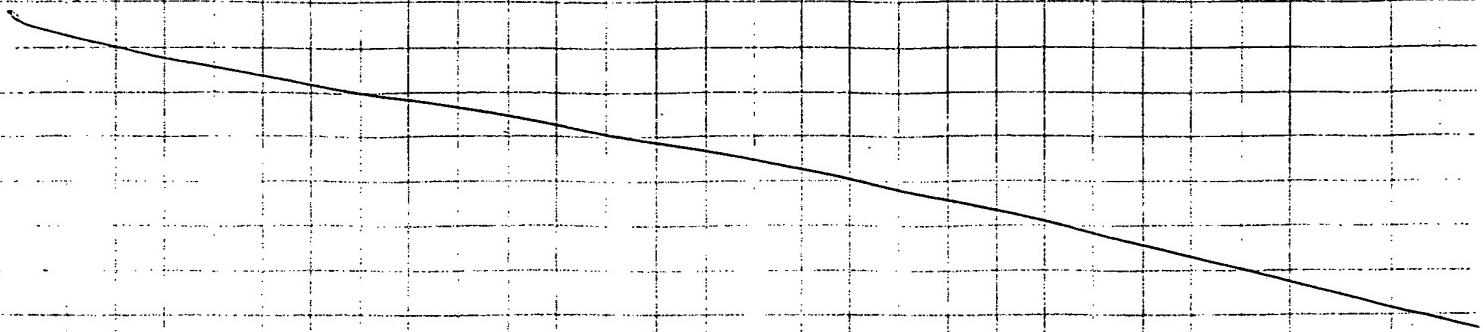
Conductivity of Lead 2.8 mS - after ~8 hrs of dialysis

Notice a small precipitate matter in dialysis tube -
spin down in SS-34 - 18,000 x g - 10 minutes -
save pellet: small + white -

- ① Load - 21 mL of sample - .75 mL/min - collect PT-7.5
- ② Wash - 2 V_t of Buffer 1 - collect 8 mL fractions 1mL/min
- ③ Gradient - Buffer 1 to Buffer 2 - 25 mL/min pH 7.5
 - 8% glycerol
 - 5 mM Tris
 - .1 mM PMSF
 - 2 M KCl
 10 V_t - 400 mL gradient (linear) - 1 mL/min - collect 7.5 mL fractions -
- ④ Wash w/ 2 V_t Buffer 2 1mL/min - 7.5 mL fractions -

Let column run O/N -

Note: Next time gradient should be much shallower - 1M KCl -



To Pag N

Witnessed & Understood by me,

Date

Invented by

Dat

- Mary Long

4/5/95

Recorded by

03/31/95

56

Project No. 20222

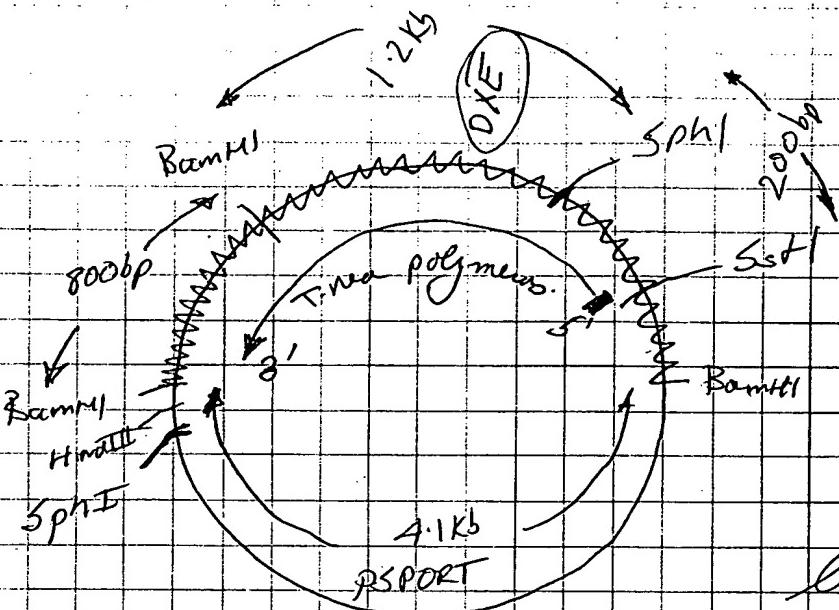
Book No. 3884

TITLE

T. neapolitana 30M

From Pag No. 33

February 7, 1995 (Tuesday)



I can clone &
Sph1 fragments
into m13mp19
and test m13
determining which
direction is less
suited.

I can subclone the
frag into an expression
vector with Sph1/HindIII.

DIGEST SCHEME

T-neo
(*λ*ZFnefue)
m13mp19

(Ready)	H2O	21 μl	✓	20 μl	✓	9:42 am →
	10X RFE	3	✓	3	✓	
DNA		3	✓	3	✓	
[CMA105]	(Young) Sph1	3	✓	3	✓	
(O-type) CAP		0	+	1	↓	9:46 am →
Total.		30 μl		30 μl		

10

Run on 0.8% Agarose Gel at 75 Volts

To Page

Witnessed & Understood by me,

Date

Inv nt d by

Dat

M. Longo

2/16/95 Recorded by

2-7-95

T-neapolitana 50M

Project N. 2022
Book No. 9884

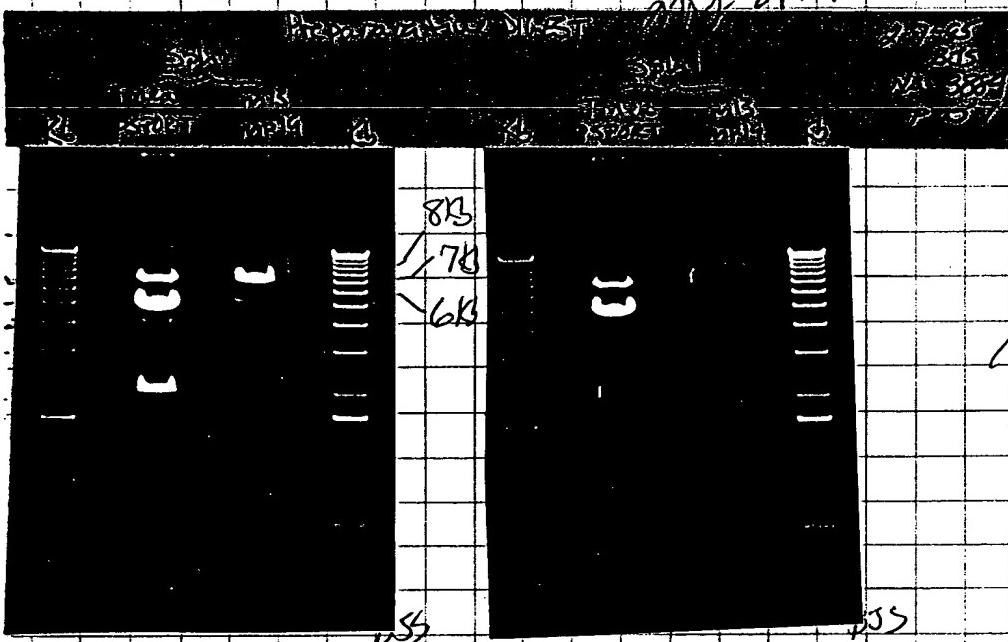
57

B N .56

0.8% Agarose Gel (1X TAE), 75 V 16

February 7, 1995 (Tuesday)

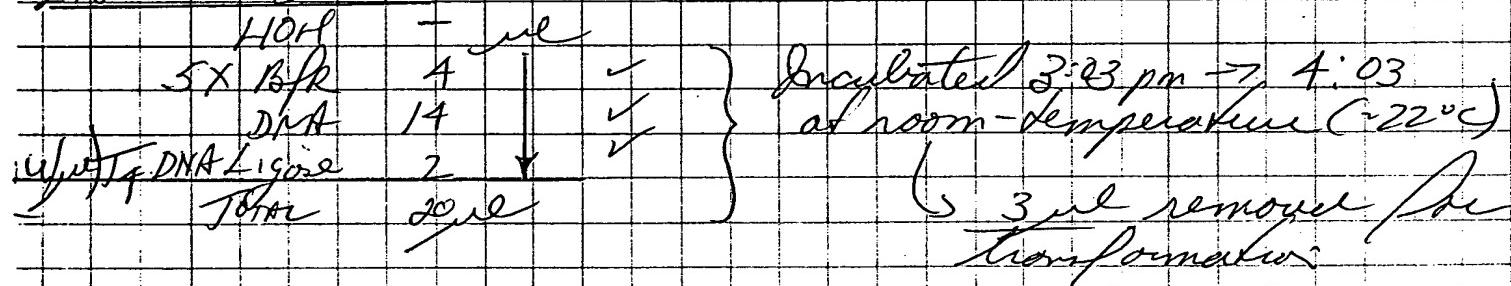
2/16/95



Bands extracted from the gel and the DNA purified away from the agarose using Gene Clean II described by B10-101.

DNA eluted in 14ul H2O

LIGATION SCHEME



152 F' IQ Competent Cell Transformation
0.1ul competent cells + 3ul ligation (see above)
2 min on ice, 35 seconds add 42°C water bath
1.0nd 90% agarose gel to LB + No Antibiotic plates in
4ml 0.7% Top agar + 100ug 2% X-gal + 100mM IPTG
incubated 16 hours at 37°C incubator

To Page No. 58

I & Understood by me,

Date

Invented by

Date

Nay Songo

2/16/95

Recorded by

2-7-95

IPMC9 / Tag + D.V

age No. _____
 year: Tag 1 U + Deep Vent different amount in PCR mix
 PMc9.

Deep Vent buffer 2.2X Klenow buffer.

1U x buffer	1.0	1.0	200 pmol dNTP
dNTP	2.2	2.2	1 μM primer
Mg	-	2.2	200 μg template
primer 1	1.1	1.1	μg 2 mm
2	1.1	1.1	
Temperature	4.4	4.4	
H ₂ O	895.6	873.6	

added 1 U Tag in 1 μl in 1X buffer.

added different amount of deep vent in 2 μl in 1X buffer
 either D.V or RT buffer
 * Tubes.

Tag	Deep Vent	Deep Vent buffer	K.T. buffer
0	0	1	23.3
1	0	3	24.2
1	.001	4	25.2
	.005	5	26.2
	.01	6	27
	.05	7	28
	.1	8	29
	.5	9	30
	1	10	31
	2	11	32
		12	33
		13	34
		14	35
		15	36
		16	37
		17	38
		18	39
		19	40
		20	41

94°, 3
 10 (94°, 30", 56°, 30", 72°, 30")

20 42

1 : 0.01 mix

started the cycling file instead of step file - 2 cycles done
 before changing to step file. To Page No. _____

seen & Understood by me,

Dat

Invented by

Dat

11/21/94

Recorded by

11/22/94

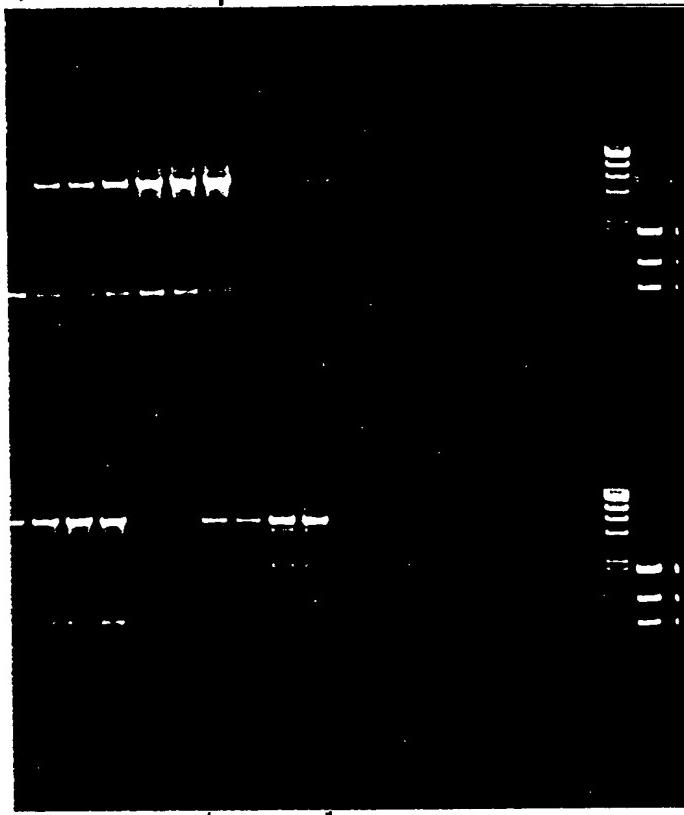
J. Sreenan

Project No. _____

Book No. _____

TITLE _____

From Page No. _____



1 1:00 | 1:01 | 1:01 → 1:02

1:005 1:05

? maybe didn't add any Tag - make premix w next
- mixing still there - check annealing temp.

- try again with new D.Vent.

Increasing D.V over 0.05 = having D.V alone
Tag effect nil.

discarded 12/19/94

Tag : dependent
titration

← D.V buffer - expt
1:0.01 ok.

← R.T. Buffer can go
up to 1:0.05 but
this can f D.V more
mis mixing

Witnessed & Understood by me,

Dat

Invented by

Dat

Recorded by

11/29/94

R. Abramson

11/29/94

To Pag N

TNE

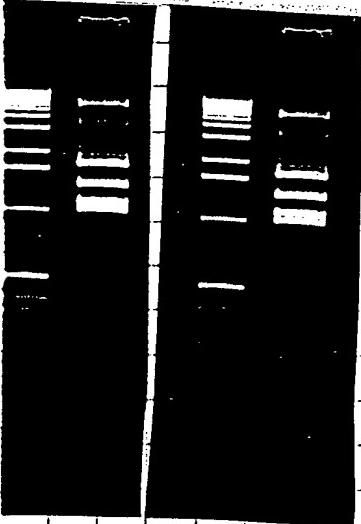
Page N. _____
1/95

7/14/95 put TNE 35% / B.SPLIT ETOH ppt. → Dissolved in 20.vl 1x1
but then 2 vL of H3 (10v/v) was added 37°C - 1 hr
applied to 1 lane of a 1% CMC agarose gel.
Gel run at 180V.

1/95 PRACTICE 35%
B.SPLIT/E3

LX
7/20/95 -

cut the 200 bp frag out &
freeze at -20°C.



200bp frag

7/17/95

Used the ^{over} phenol extraction method to purify DNA.
Dissolved in 10.vl TE.

To Page No. _____

signed & Understood by me,

Date

Invented by

Date

Lili Xu

7/20/95

R recorded by
Amy Longo

7/18/95

72

Project No. _____
Book No. _____

TITLE

SDS gel Thermostable pols.

From Page No. _____

30' ice 10' microfuge at 4°C , remove supl
Vortex pellet in ice cold acetone, microfuge 10' remove supl,
dry 57°C 25', resuspend in 60 μl x cracking supl

Witnessed & Understood by me,

Date _____

" 29/94

Invent d by

Recorded by

Date

10-25-84

g No. _____

9

10

11

m/s

TNC

native
Taq

Tne

mW

immobilized as per

P148, 6

start 29 mA at 1:35 pm
 gel as per P140, 6

To Page No. _____

ed & Und rsto d by me,

Dat

Inv nted by

Date

envelope & Postage

11/29/94

Recorded by

10-25-94

PNc9 / D.V.

Pag N .

try to amplify PNc9 with Deep Vent alone
bred in Deep Vent buyer only.

10x buyer	100
dnTP	20
Mg	-
Primer 1	10
2	10
Template	4
H ₂ O	846

200 µg dNTP
2 mM Mg
1.2 µM primer
200 µg Tengibak'

94° 30°

30(94°, 30°, 56°, 30°, 72°, 30°)

0	1	2
.001	3	4
.005	5	6
.01	7	8
.05	9	10
.1	11	12
.5	13	14
1	15	16
2	17	18

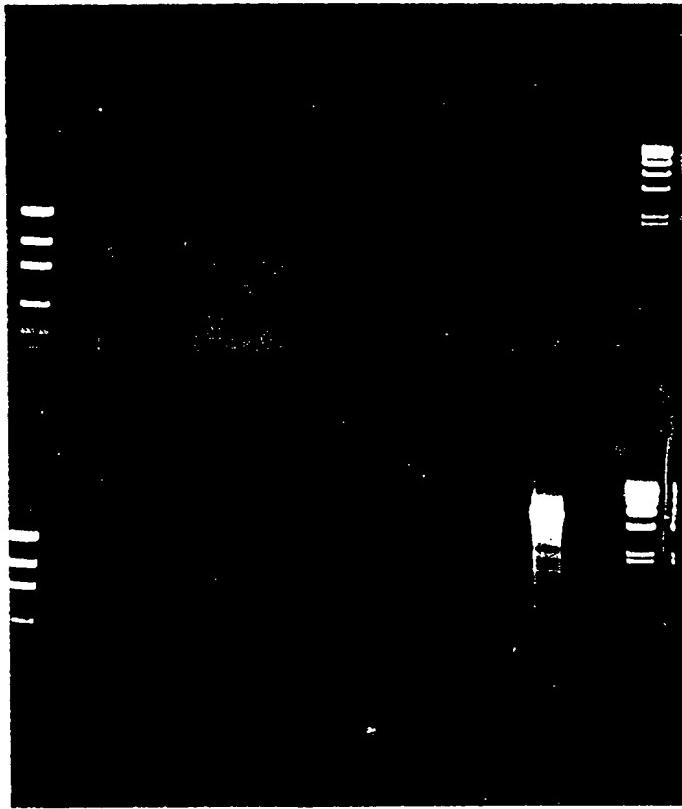
result: Once again

Deep Vent alone by itself

didn't amplify anything

with this DNA primer.

Say the other set to
confirm.



To Page No. _____

Issued & Understood by me,

Date

Inventoried by

Date

Recorded by

A. Sitaraman

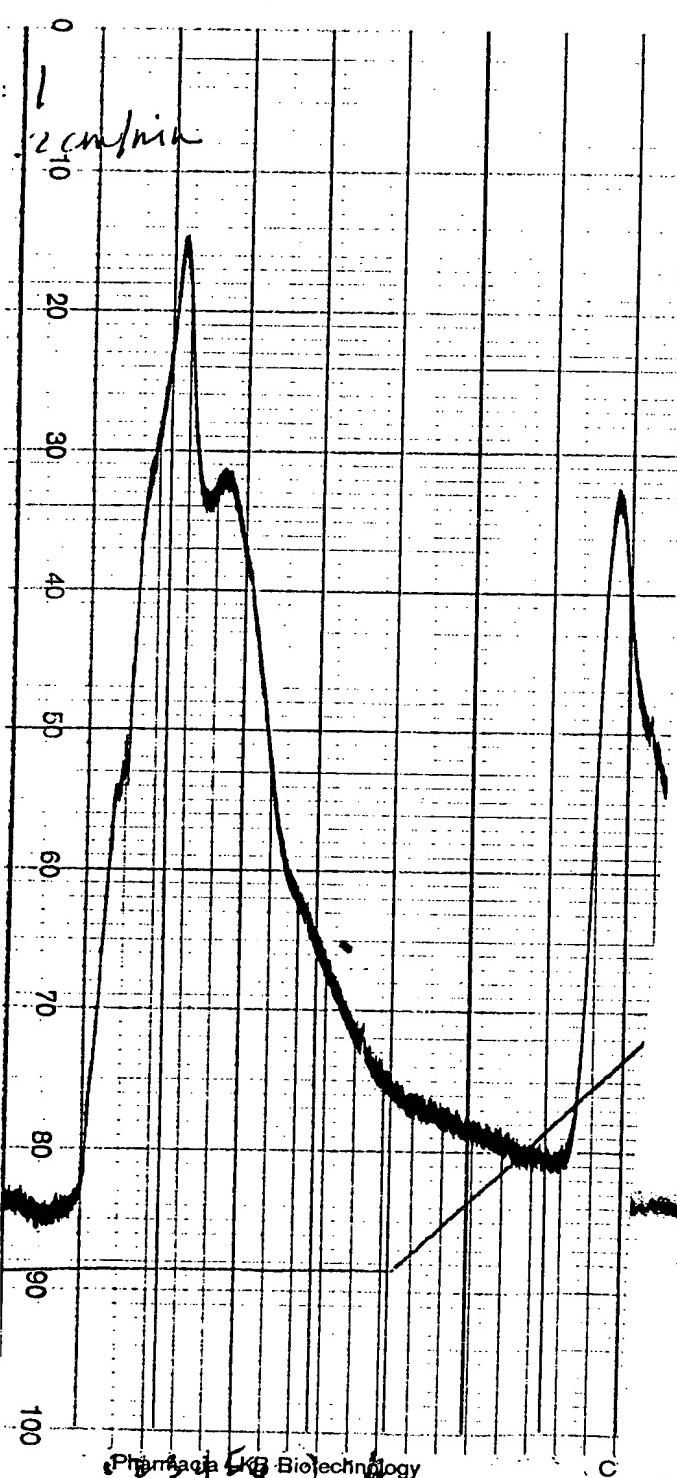
11/22/94

Project No. _____

Book No. _____

111

Bag No. _____



3/3/95

NY
4/15/95

To Page No. _____

Read & Understood by me,

Date

Entered by

Date

May Longo

4/15/95

Recorded by

05/31/95

Project No. _____
Book No. _____

TITLE 4 column Activity

From Page N _____

SAM CPM1

1	135612.00	lead
2	310.00	
3	460.00	2
4	512.00	3
5	386.00	4
6	308.00	5
7	1118.00	6
8	960.00	10
9	546.00	15
10	420.00	16
11	1368.00	17
12	6588.00	18
13	45516.00	19
14	70278.00	20
15	98796.00	21
16	91534.00	22
17	109058.00	23
18	129224.00	24
19	73534.00	25
20	32032.00	26
21	13662.00	27
22	3166.00	28
23	2848.00	29
24	1910.00	30
25	1508.00	31
26	1426.00	32
27	3168.00	33
28	1278.00	34
29	840.00	35
30	516.00	
31	119806.00	
32	121684.00	
33	123400.00	
34	26.00	
35	44.00	
36	50.00	

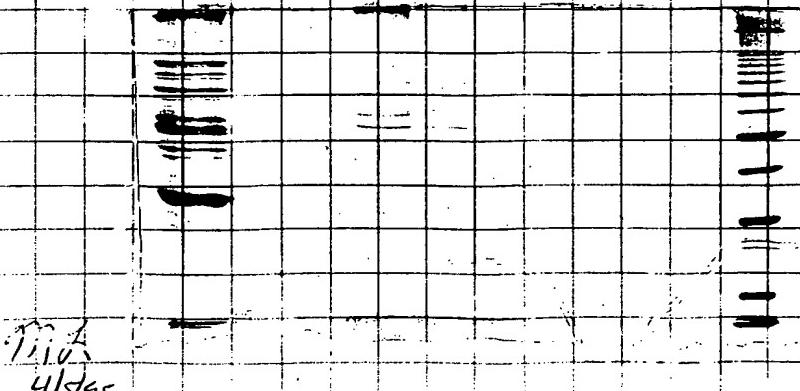
4/5/95

POOL

dry + count

Pool dialyzed S/N (Sat). 104/01/95

Gel of New Nisoya Fractions



4/5/95

To Page N

Witness d & Understood by me,

Date

Invented by

Date

Mary Long

4/5/95

R e c rded by

E. Ryan

03/31/95

7 column Bradford's → PAGE

Project No. _____

Book No. _____

113

ag N -

Conc (mg/ml)

11.274956
0.307192
1.065601
0.819939
3.081949
0.385722
0.375194
0.285862
0.329252
0.368813
0.432621
0.980098
0.798244
0.419222
0.177069
0.069870

Cude
Heat Kill
DEI
AS Sup
A Load
17
18
19
20
21
22
23
24
25
26
27

- too Not dilute enough

3/31

∴ PAGE -

1 2 3 4 5 6 7 8 9 10
nde Heat DEI AS M Load 17 18 19 20
Kill Sup. M

1 2 3 4 5 6 7 8 9 10
20 21 22 23 24 25 26 M
27 28 29 30 31 32 33 34 35 36 M

✓

3/31

03

To Page No. _____

sed & Understood by m ,

Date

Invented by

Date

Mary Foyse

4/5/95

Recorded by

03/31/95

+ME

Project No. _____
Book N . _____

Page N -

Goal: To clone the T-NE 35 Fy (mut) into pTTC99A or a similar vector.

New Scheme

PUC TNE 35 Fy → H3 → Klenow → Sph I → Scl puc
(~ 5.1 kb) 2 kb
frag

Clone into the
Sma I / Sph I size of pTTC19.

pTTC19	4	= ~ 2.5
10x R4	4	
H ₂ O	30	
Sma I	2	
10uL	40	

30°C - 1 hr.
LX
8/3/95

1 PUC TNE 35 Fy cut w/ H3
2 pTTC19 cut w/ Sma I

Cuts look good

40

Sph I
10uL

42

37°C - 1 hr.

PUC TNE 35 Fy / H3

10x R2

10mm dNTP mix

Klenow

40

20

2

0.5-1

52.5

ice 5'

EDTA to 20mL

phenol extract
Taq/gold/2D gel

read & Understood by me,

isbnr

Date

8/3/95

Invented by

Recorded by

Date

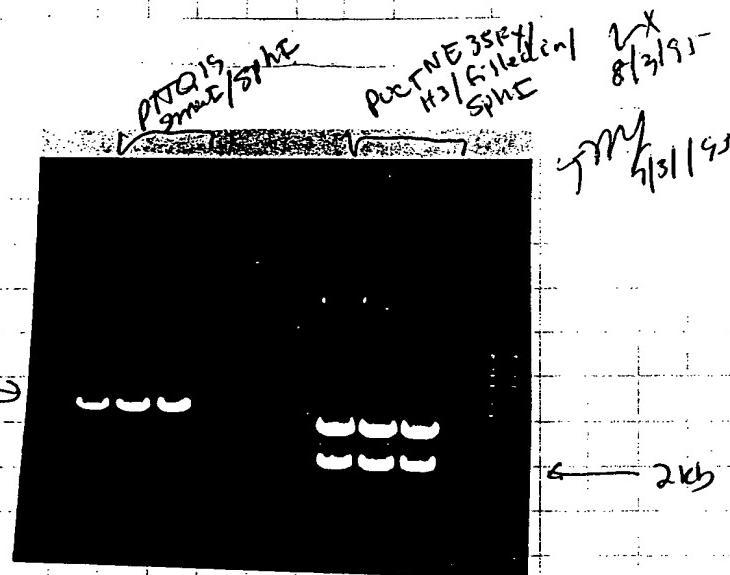
7/31/95

Project No. _____
Book No. _____

TITLE TNE

From Page No. _____

pUC TNE 35SFY / H3 | filled in → resuspended in 40 μl 1X RE
2-1 of 100μl SphI
37°C - 1 hr.
Applied to c. 0.9% agarose gel.
Gel run at 180V.



cut bands out +
freeze at -20°C

Gene clean the frag as usual.

Dissolved in 10 μl H2O.

Applied 10 μl to a 0.9% agarose gel.
Gel run at 180V

1X
8/3/95



gym 4/31/95

- 1 pTTA15 / SphI / SphI
- 2 2 kb H3 / Filled in / SphI frag
from pUC TNE 35SFY

~ 10 ng/μl = .
~ 20 ng/μl = .

Witnessed & Understood by me,

L. Ishii

Date

8/8/95

Inventoried by

W. L. Ishii

Date

8/1/95

To Page 1

TNE

Project N _____
Bk No. _____

183

sig N _____

lig

TQ19 / Smal I / Sphe I .003 pmol/.1
 kb 113 / Killed in / SpnI .015 pmol/.1
 5X 1/5000 buffer

H₂O

Ligase (10)

2
1.5
1
4
125
1
20-1

RT - 30 min

Jason xformed 2 ul of the lig in with 100-1 DTAO3 cc.
 std xform. Plated 10³, 10⁴ + 90% on get amp plates. 37°C

#2	10 ³	10 ⁴
	18	~150

picked 8 colonies into 3 mls of CG + amp/100. 37°C = ON

mp as usual. Disolved in 5 ml TE.

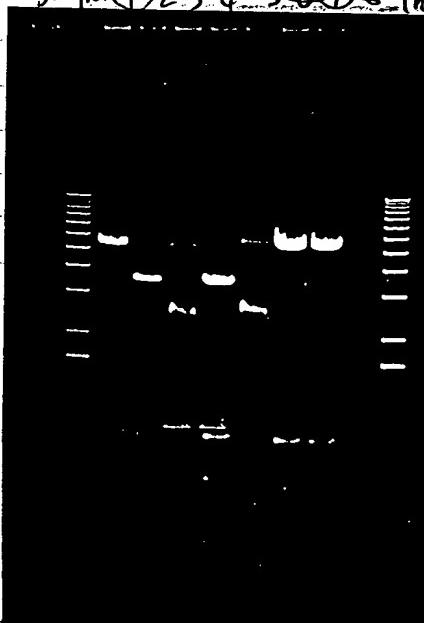
mp	3
1D(R2)	2
H ₂ O	13
BphI	1
EcoRI	1
	10

sub PCTNE 35 PY mot into
 Smal / Sphe I size of pTT019
 clones cut & sonicated
 st 1kb (1, 2, 3, 4, 5, 6) < 1kb

AN
8B/85

37°C - 1 hr.

Applied to a
 0.7% agarose
 gel. Gel
 run at 100V



To Page No. _____

Signed & Understood by me,

Fisher Xn

Date

8/3/95

Invented by

R. corded by
John Longo

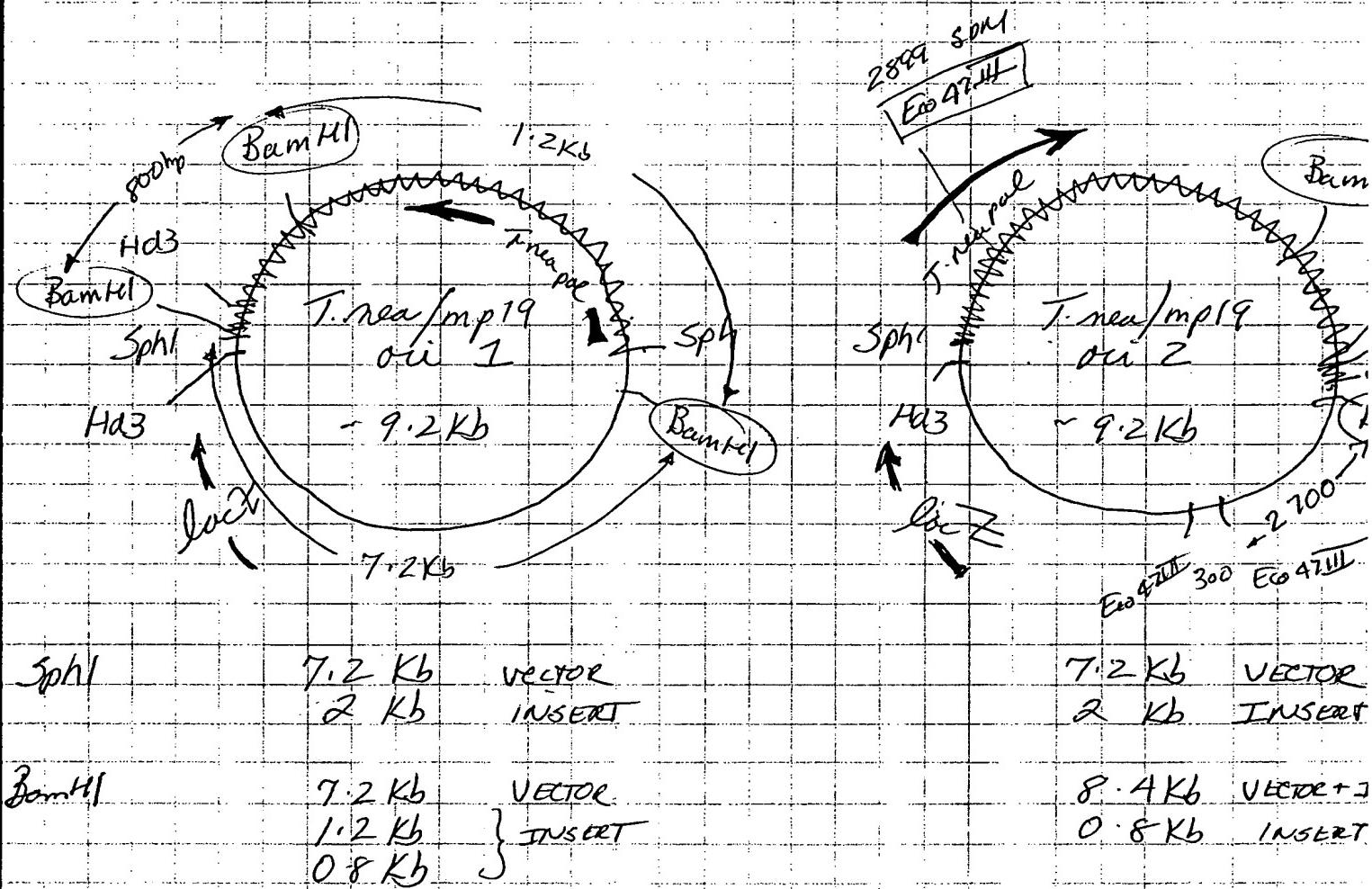
Date

8/13/95

From Pag No 57

February 8, 1985 (L)

- I added 200 μl of DMSO/F IQ lawn cells to 10m. circle brown.
- Added 1ml of the cells to 8 glass tubes
- Each tube was inoculated with a clear plug and incubated at 37°C (8:00 am →



Add *T. nea/mp19NT* as a positive control for both dysk

Witnessed & Understood by me,

Date

Invent'd by

Date

Mary Longo

2/16/85

Recorded by

Frank Schmidt

To Page

2895

Teneapalitoxen SDM

Project No. 20227
Book No. 3884

59

ag No 58

February 8, 1995 (Wednesday)

DIRECT SCHEMES

		PER RXN X 9 = COCKTAIL	
	HOM	7 μl x 9 = 63 μl <input checked="" type="checkbox"/>	
React 6) 10x Bfr	2 x 9 =	18 μl <input checked="" type="checkbox"/>	
	DNA	10	
(10μg/u) Spn	1 x 9 =	9 μl <input checked="" type="checkbox"/>	
	Total	20 μl	90 μl

For Teneapalitoxen
control add

T10 E1 20 μl
DNA 2 μl
Tome 22 μl

✓

		PER RXN X 9 = COCKTAIL	
	HOM	7 μl x 9 = 63 μl <input checked="" type="checkbox"/>	
React 3) 10x Bfr	2 x 9 =	18 μl <input checked="" type="checkbox"/>	
	DNA	10	
(10μg/u) BomH1	1 x 9 =	9 μl <input checked="" type="checkbox"/>	
	Total	20 μl	90 μl

add 10 μl to reaction

Confined on page 1 of Notebook 3966

Axon - Patel Gel photo

T Page No.

seen & Understood by me,

Date

Invented by

Date

May Tong

2/16/95

Recorded by

Bruce J. Schmidt

2-8-95

ig N -

Con'd. from 3884 NB

2/18/95 wed

MINI PREP DNA

- Cfg 500 ul of cells for 1 minute in an eppendorf (cfg centrifuge)
- removed supernatant and resuspended pellet in 100 ul of 1X PEGI (SI)
- added 200 ul of alkaline - SDS mix
- placed the tubes on ice for few minutes (3-5 min.)
- added 150 ul of 7.5 M Ammonium Acetate
- Mixed the tubes by inverting
- cfg the tubes for ~ 7-10 min.
- transferred 400 ul supernatant to the new eppendorf tube
- added 800 ul of ethanol to supernatant. Mixed tubes.
- incubated the tubes for ~ 2 min. Spin.
- dissolved pellet in 50 ul of TE + RNase A
- applied 5 ul to a 1% agarose gel.



SI = 0.9% glucose

25 mM Tris HCl (pH 8.00)

10 mM EDTA

alkaline - SDS mix = 1% SDS

0.1 N NaOH

To Pag No. _____

Assed & Understood by me,

Date

Invented by

Date

Recorded by

4/12/95

70

Proj ct No. _____

Book No. _____

TITLE _____

From Page No. _____

Tf(min)

1.33

4

Agarose

10 5 10 15 20 25 30 36 10 5 10 15 20 25 30 36 cycles

O/N exposed 100-100,000 gray

Result: no 13.5 K6 product - maybe too much Mg⁺ with hot primer

nonspecific smear also seen with EtBr (P.69) is cold in auto and after above

To Page N

Witnessed & Understood by me,

Date

11/29/94

Invented by

Rec rd d by

Date

10-27-94

Deborah Bolay

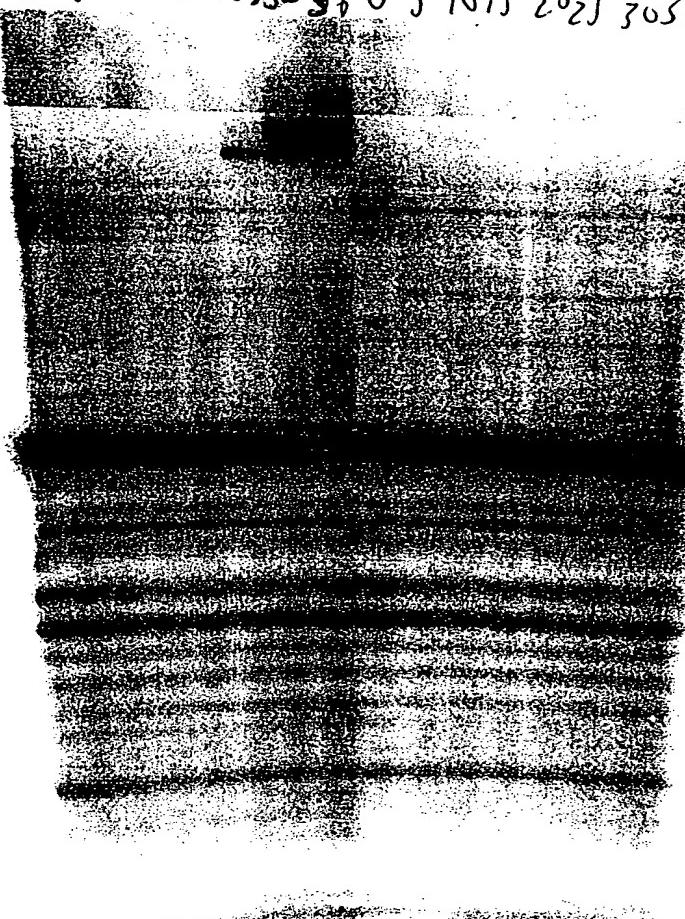
ag N .

PAGE

Tfl min 1.33

4u

0 5 10 15 20 25 30 35 0 5 10 15 20 25 30 35 cycles



O/N
exposure

100 -
100, 100
gray

Results:

only slight degradation (< 1 nt/primer) after
36 cycles
note formation of primer dinner cycles 25-36
but only for block Tfl

To Page No. _____

ssed & Understood by m ,

Date

Seema Golay

Invented by

Rec rded by

Date

(10-27-94)

14

Project No. _____
Book No. _____

TITLE Q6SDM - Tsohaes -

rom Pag No. _____

Used DEPC treated water to make all buffers from
water on out -

Washed column + column matrix extensively with
5 M NaOH -

Poured a 40 mL Q6SDM - 8cm x 2.5cm - col
Wash w/ 5N NaOH
Wash w/ 1L of DEPC treated sterile H₂O
Wash/ & Equilibrate w/ Buffer A

Buffer A -

Buffer B.

25mM KPO₄, pH 7.2

25mM KPO₄

pH 7.2

10% glycerol

10% glycerol

10 mM KCl

800 mM KCl

5 mM Bme

5 mM Bme

.1 mM PMSF

.1 mM PMSF

↓
after conductivity 3.2 mS

50mS

Sample conductivity 4 mS - dialyzed in buffer A - ~ 27.5 mL - col
from dialysis

Program -

Load 0.5 mL/min.

Wash w/ 120 mL of Buffer A 1mL/min - collect 7.5 mL fractions 2.

Gradient - 400 mL linear gradient Buffer A - Buffer B - 1mL/min " 10

Wash w/ 120 mL of Buffer B 1mL/min collect 7.5 mL fractions -

To Page N

Witnessed & Understood by me,

Date

Invented by

E. Flynn

Date

May Tongo

4/15/85

Recorded by

4/12/95

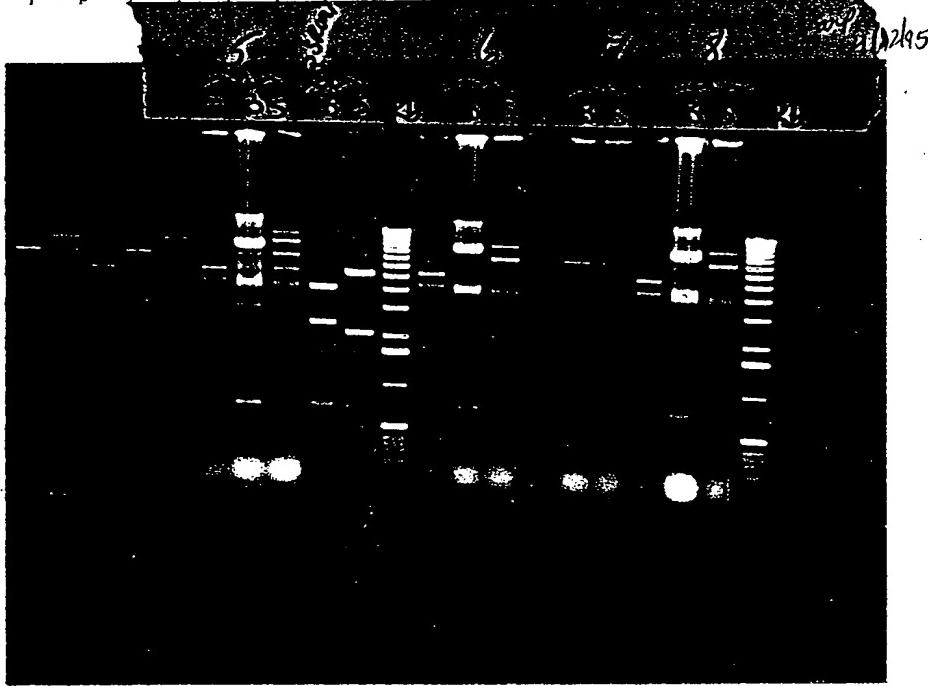
Fig N. —

Con'd from 3884 NB

218/95 wed

MINI PREP DNA

- Cfg 500 μ l of cells for 1 minute in an eppendorf (cfg centrifuge)
- removed supernatant and resuspended pellet in 100 μ l of 1 X PEGI (SI) (saved)
- added 200 μ l of alkaline - SDS mix
- placed the tubes on ice for few minutes (3-5 min.)
- added 150 μ l of 7.5 M Ammonium Acetate
- mixed the tubes by inverting
- cfg the tubes for ~ 7-10 min.
- transferred 400 μ l supernatant to the new eppendorf tube
- added 800 μ l of ethanol to supernatant mixed tubes
- incubated the tubes for ~ 2 min. spin
- dissolved pellet in 50 μ l of TE + RNase A
- applied 5 μ l to a 1% agarose gel.



Project No. _____
106 11/22/94 Book No. _____

TITLE MEG / new primers.

From Page No. _____

* AAT II * 1 : Gc e ACC TGA C G T C T A A G A A A C C A
now du 31 mer. TAT TAT e

* AAT II * 2 : G T T T C T TAG A C G TCA G G T G G C ACT T T T
now du 29 mer.

* AAT II * 1 : Gec ACC UGA CGAU CUA AGA AIC CAU TAT
du 31 mer.

* AAT II * 2 : GTT TCT UAG ACG UCA GGU GGC ACC
du 29 mer. TTT

Purpose: To try to amplify pycg with these new primers.
increased the annealing to 58° from 55° (previous exp.)
enzyme: Tag alone (1U) primers: 1 now du 2
Tag + Degravit (1 + .01) du
D.V. (0.5 + #) 3 du - all
done in duplicate.

12 Rx / enzyme - use premix media.

4 1 du + 2 no du

5. 2 du + 1 no du

- Dep. Venk buffer was used thru out.

Tag:	buffer	60
	dNTP	12
	Tempolyse	2.4
	enzyme	2.4
	H ₂ O	403.2
		<u>180</u>

Tube # 1 - 11

200 μM dNT,
200 μg Tempolyse
1 μM primer
2 mM Mg²⁺
comes w. buffer

40 μl / Rx - added def. primer in 10 μl -

To Page No.

Witnessed & Understood by me,

Date
11/22/94

Inventoried by

Date

Recorded by

11/22/94

J. K. Laramore

ag N

primers old) $\frac{dV}{dU} = 20 \times \frac{25}{10} + 10 \text{ ml } \downarrow \text{ each primer at } 180 \text{ ml } 1/20$ (100 μM)

primers new) $\frac{dV}{dU} = \frac{1}{2}$ equivalent amount of each other 2 different combo
1 dU + other men dU $50 + 50 \text{ ml } (10 \mu\text{M} \text{ each})$

D.V.: buffer 60 D.V alone $0.5 \mu\text{l}$ 1 U
dNTP 12

01) Temp 2.4

pm Enzyme 12.0 $(1 \cdot 01/\lambda)$
1/20 398.6

400.0

1

buffer 60 60
dNTP 12 12

Temp 2.4 2.4
enzyme 2.0 6.0

402.1 399.6

1

ube #

12 - 21

23 - 33

33 - 44

1, 22, 33 or 44 w/o any primers.

cycling: $94^{\circ}, 3'$

$30(94^{\circ}, 30", 50^{\circ}, 30", 72, 2') \rightarrow 4^{\circ} \text{ soak}$

samples thrown out 12/19/94

To Page No. _____

ssed & Understood by me,

Date

11/28/94

Invent d by

R corded by
K. Subramanian

Dat

11/28/94

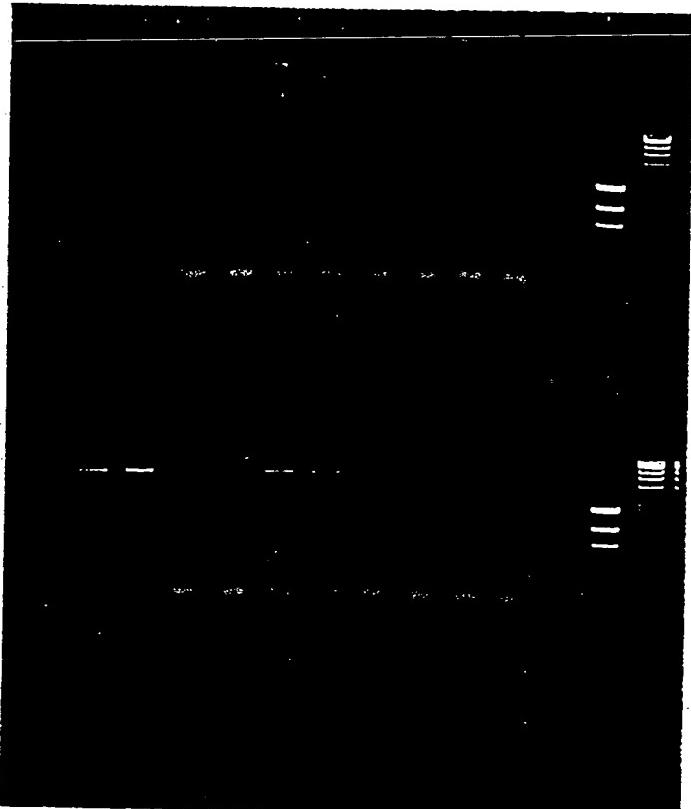
Project No. _____

Book No. _____

TITLE _____

in Page N. _____

Tag:



$N + N + O + - / + - / +$ ^{no} primer

Tag + D.V 1:0.01:

Deep red as usual
darker red →

With old d.v primer shows
for the first time.

Why more primer darker
with d.v than with
new d.v?

Result:

- Even with Tag problem with product at the annealing temp.
- F/R - red product as slightly with Tag, mix with T + D.V
- old primer at 58° did work with Tag.
- with Tag + D.V mixer all work good with old primer new mixer product with T + D.V
- mismatch didn't work at all.

D.V 0.50



Page No. _____

ness d & Understood by me,

Date

11/23/84

Entered by

Recorded by

Date

11/23/84

TNE

Project No. _____
Book No. _____

183

ag N _____

Lig

TQ19/SmaI/SphI .003 pmol/.

xb H3/Cold in SphI 0.15 pmol/.

5X 150μl buffer

H₂O

Ligase (10)

2

1.5

1

4

125

1

20.1

RT - 30 min.

Jason transformed 2.1 of the lig in with 100 μl 34/0.3 cc.
std & form plated 10% + 90% on yeast agar plates. 37°C

2N

10^b 90^b
#2 18 ~150

picked 8 colonies into 3 mls of CG + ampi. 37°C - ON.

5S

mp as usual. Dissolved in 50 μl TE

and put TNE 35 μl mol into

SmaI/SphI 5.4 μl of pTQ19

clones cut SmaI/EcoRI

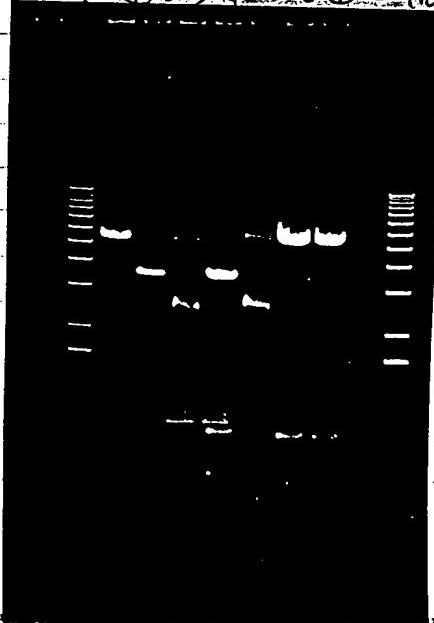
st 1kb(1) 2 3 4 5(6) 6 1kb

AN
8B/55

mp 3
100μL 2
H₂O 13
BphE 1
E.coli 1
10

37°C - 1 hr.

Applied to a
0.7% agarose
gel. Gel
run at 150V



To Page No. _____

seen & Understood by me,

Lisha Xin

Date

8/7/95

Entered by

Recorded by
Cathy Long

Date

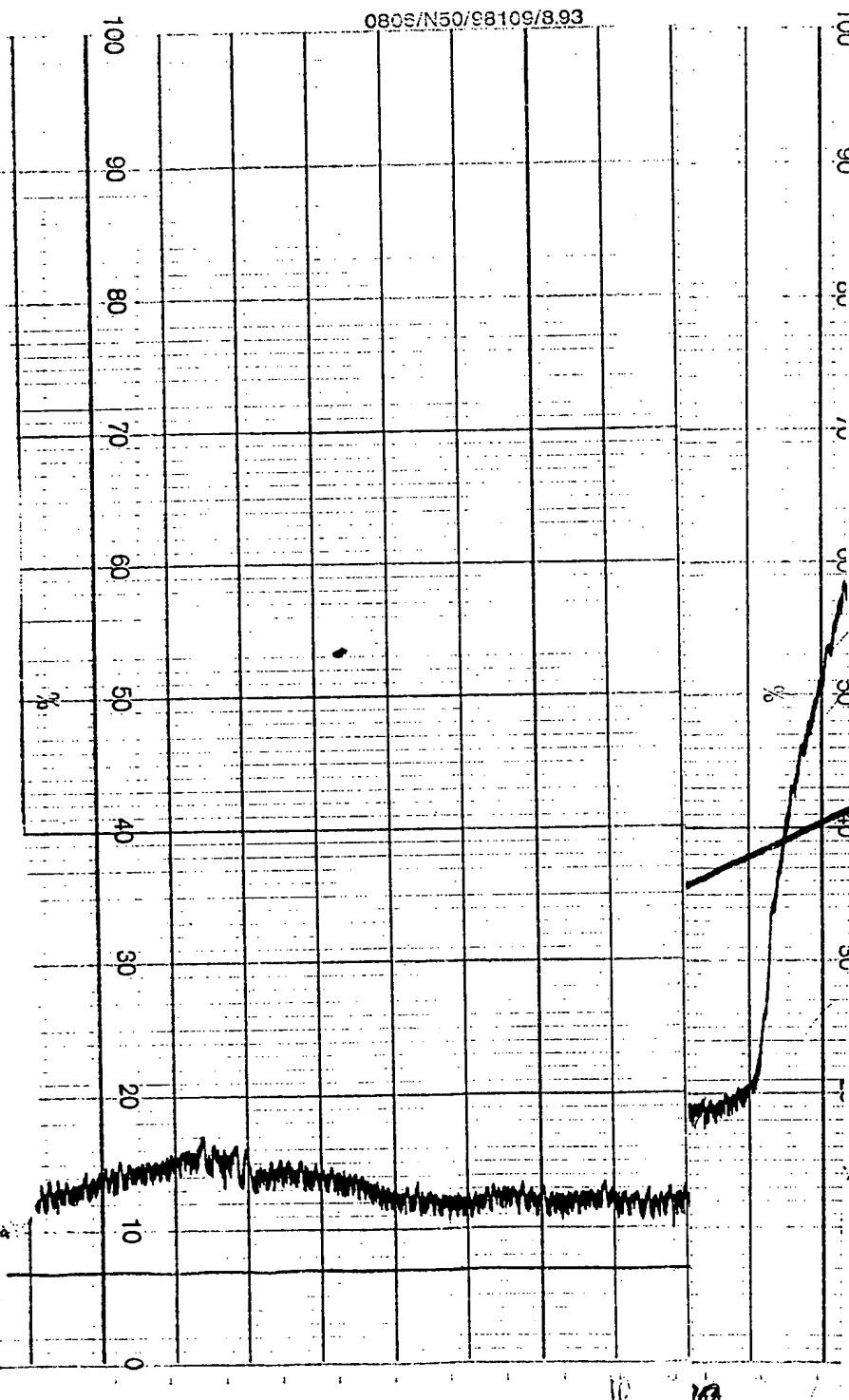
8/13/95

Project No. _____
Bk No. _____

115

Heparin Q 650 - The Purification

Page No. _____



27 04/03/95

9771
4/5/95

To Page No. _____

S & Understood by me,	Date	Invented by	Date
May Longo	4/5/95	E. Flynn	04/03/95

Recorded by

Project No. _____

Book No. _____

TITLE _____

2 From Page No. _____

2/9/95 Th

Purification of m13 ssDNA

1. Cfg 1.0 ml of *infected cell culture for 2 min. (1 to 5 m)
2. Transferred 800.0 μ l to the new tubes
(Pellet was saved for isolation of RF DNA)
3. Cfg supernatant again to remove any residual cells
4. added 200.0 μ l of 20% PEG + 1.5 M NaCl. Vortexed
5. Incubated tubes at room temperature for 5 min.
6. Cfg tubes for 5 min. El discarded supernatant (sup.)
7. added 200 μ l of *TE El vortexed really good.
8. Cfg for ~ 1-2 min. (to remove any residual cell debris)
9. transferred sup. to the new tubes. (RNaseA can be added here)
10. added equal vol. of Phenol / chloroform / isoamyl alcohol (25:24:1) Mixed well.
11. Cfg 5 min.
12. removed the aq (upper) layer to a new tube (be very careful)
13. added 1/10 vol. of 3M NaAc. + 2 1/2-3 vol. of 95% EtOH
14. Incubated @ -70°C till 2/14/95.

{ 20.0 μ l NaAc
600.0 μ l EtOH

$$TE(T_{10}E_1) = 10 \text{ mM Tris-HCl pH 8.0} + 1 \text{ mM EDTA pH 8.0}$$

infected cell culture = ① grew an e. coli F' strain to an OD of 0.4 in 2xYT
↓
neat pag

F' = Fertility factor: codes for tra genes on pili to allow infection of the m13 phage.
(transfer of RNA)

Cont'd _____. To Page No _____. _____

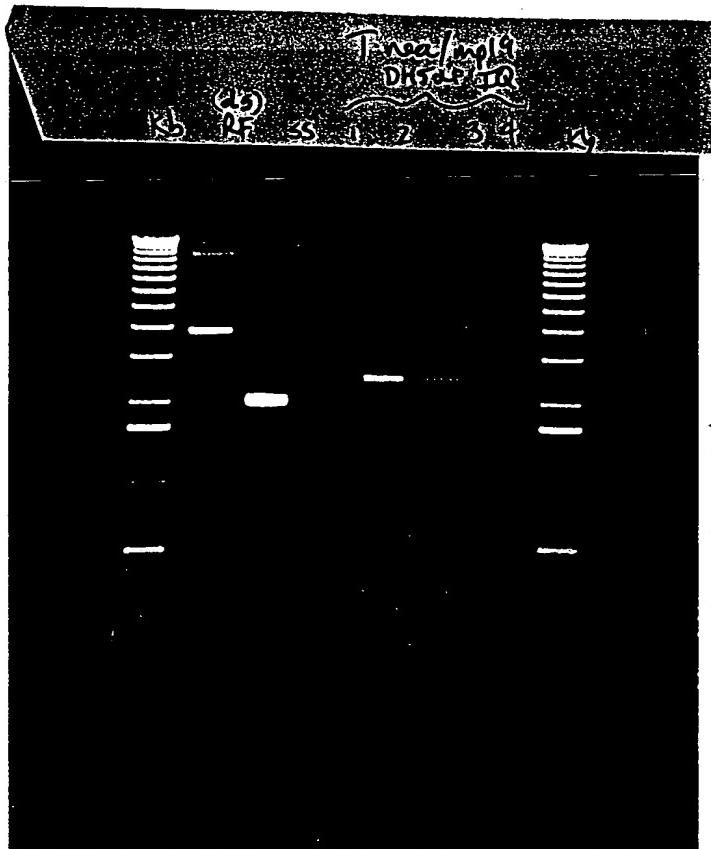
Witness d & Understood by me,	Date	Invented by	Date
<i>Deneen S. Berg</i>			
4/12/95		Recorded by	
<i>Deneen S. Berg</i>			4/12/95

Project No. _____

Book No. _____

5

age N. _____



Run 1404 ~2 hrs.

To Page No. _____

sed & Understood by me,

Date

Invented by

Date

Dolcups

4/12/95

Recorded by

4/12/95

TITLE

TNE

From Page No.

8/1/95

Lig

2
1.5
1
4
125
1
20.1

pTTQ19 SmaI/SphI .003 pmol/.1
 2 kb. H3 Filled in SphI .015 pmol/.1

5x 150μl buffer

H₂O

Ligase (10)

RT - 30 min

Jason formed 2.1 of the lig with 100.1 34103 cc.
 std xform. Plated 10% + 90% on get amp. plates.

8/1/95

10% 90%

#2 18 ~150

picked 8 colonies into 3 mls of CG + ampi. 37°C

8/3/95 mp as usual. Dissolved in 5 ml TE

sub PUC TNE 35 RY mot into
 SmaI/SphI site of pTTQ19
 clones cut SphI/EcoRI
 5 kb (2.3 v 5.0) & 1 kb

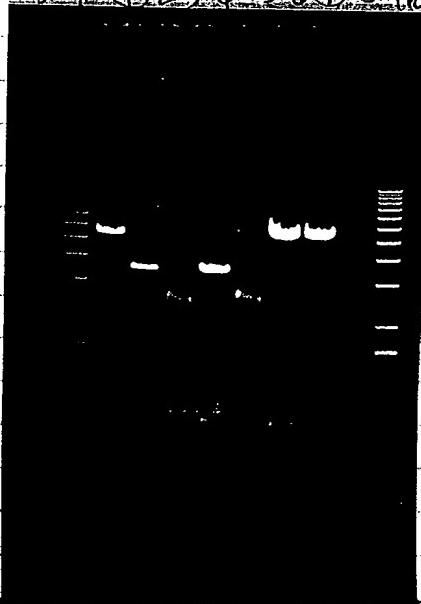
AN
81

mp	3
10xRL	2
H ₂ O	13
BphT	1
E. coli	1
	10

37°C - 1 hr.

Vector 4575
 insert 2000
 6575

Applied to a
 0.7% agarose
 gel. Run at 100V



To Pag No.

Witness d & Und rstood by m ,

Lisha Xn

Date

8/3/95

Invented by

Recorded by
 C. M. L. T. M. D.

Date

8/3/95

Annealing Temperature

PME / dif. engg / dif. primers / dif.

Book No. _____

11/28/94

tag No. _____

purpose: To check at different annealing temp. to get rid of mis priming during PME amplification

Terry checked 58°, 60°, 62°, 65°

56° gave more non specific bands than 58°. pg 102-

checked all three primer set SAII - new dv

{ " " old dv 2728 + 29

Amplified with Tag, Tag + DV, DV alone.

200 μl dNTP

1 μl primer

200 μg template

2 ml buffer (from buffer) - used deep well buffer

prepared cocktail for 80 Rx

added primers separately, adding reverse enzymes.

lane #	1 - 8	Tag	old dv	each combination
9 - 16		New dv	in duplicate	
17 - 24		" - dv	annealed at differ.	
25 - 32	1 : 0.01 DV	old dv	temp 58, 60, 62, 65°	
33 - 40		new dv	94° 3'	
41 - 48		" - dv	30 (94° 30" X 30")	
49 - 56	Deep Well	old dv	72° 3'	
57 - 64		new dv	72° 10'	
65 - 72	↓	" - dv	4° 20 min.	
	1.5 U			

To Page No. _____

Signed & Understood by me,

Date

12/19/64

Invented by

Dat

11/28/94

Recorded by

L. S. Aramann

Project No. _____

110

Book No. _____

TITLE _____

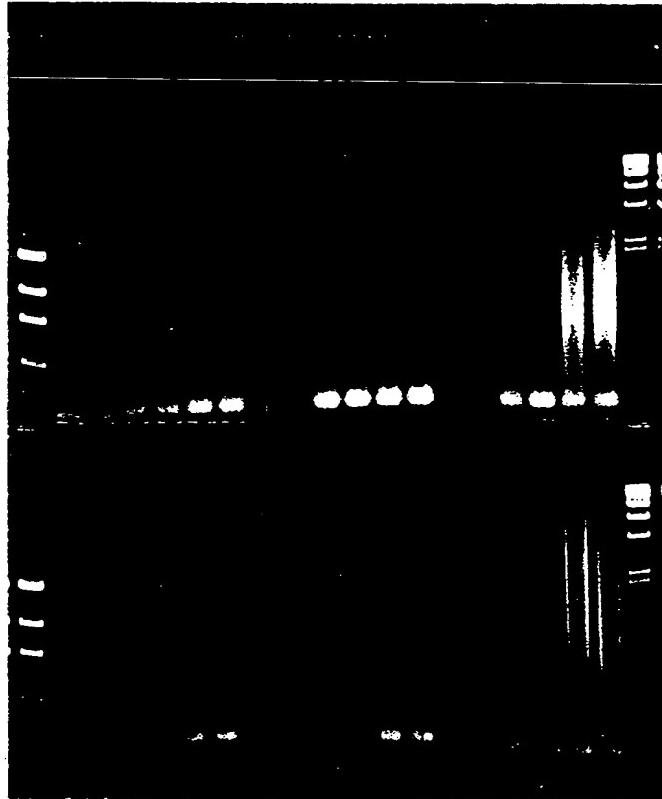
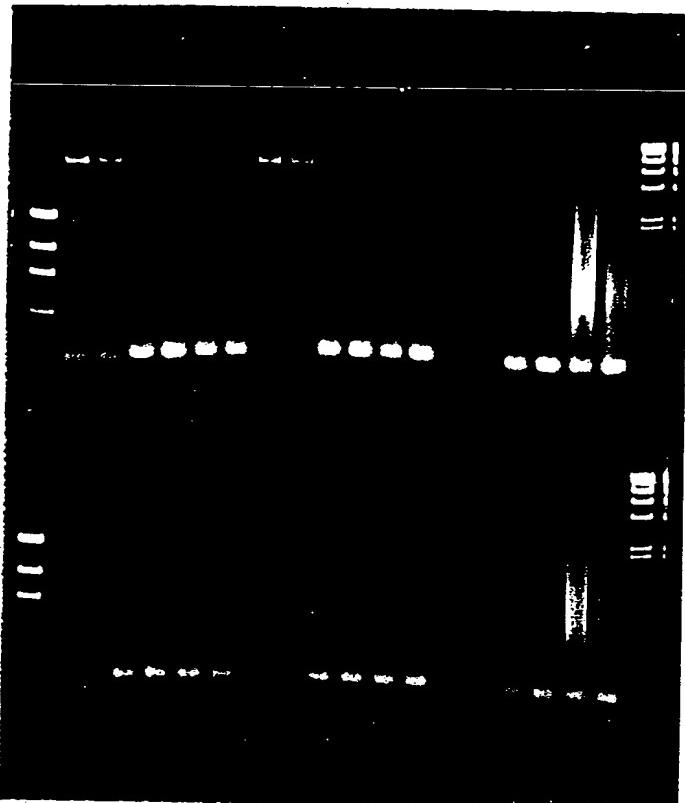
From Page No. _____

Tag DV

D.V

O-JUN-80-TU

58°



60° CW

Result: The only thing that worked,

Tag DV
all DV primers at 58°
Tag + DV

Deep Vent / non DV / at all annealing Temp seems?

All samples discarded

To Pag N

Witnessed & Understood by me,

Date

Invented by

Date

[Signature]

12/18/80

Recorded by

[Signature]

11/29/80

70

Project No. _____

Book No. _____

TITLE _____

From Page N .

Tf (min)

1.33

4

10 5 10 15 20 25 30 36 10 5 10 15 20 25 30 36 cycles

Aga

O/N exposure 100 - 100,000 gray

Result: no 13.5 KG product - maybe too much Mg with hot primer!

nonspecific smear also seen with EtBr (P69) is cold in auto rad above

Witnessed & Understood by me,

Dorothy Polans

Date

11/29/94

Invented by

Recorded by

To Page

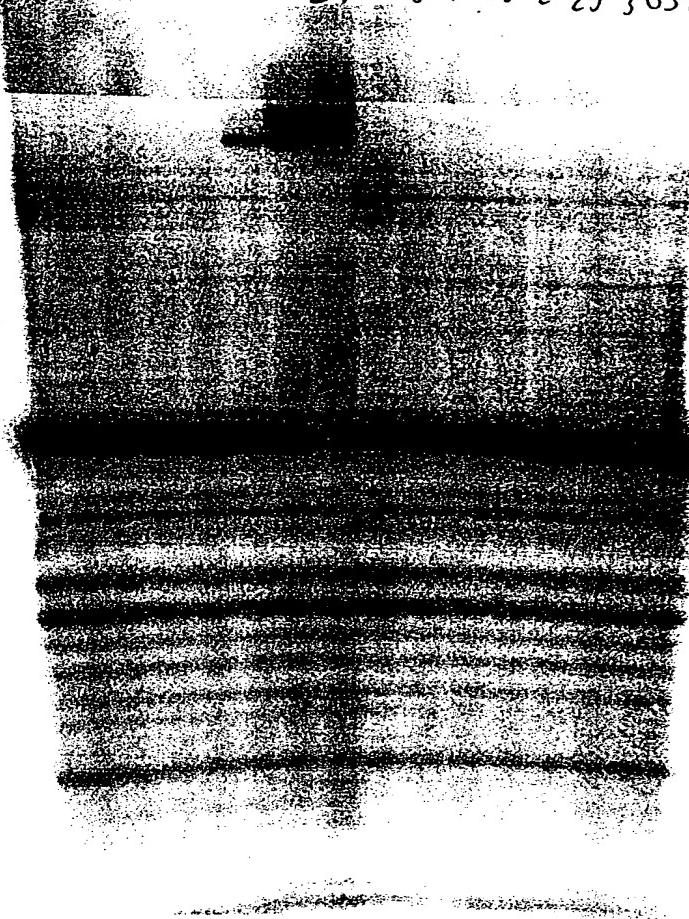
Date

10-27-94

Tf1 initial 1.33

4u

3 5 10 15 20 25 30 36 0 5 10 15 20 25 30 36 cycles



O/N
exposure

100 -
100, 100
gray

Result:
only slight degradation (< 1 nt / prime) after
36 cycles
note formation of prime dinner cycles 25-36
but only for blob Tf1!

T Page No. _____

Read & Understood by me,

James Gobey

Date

11/29/94

Inv nt d by

[Signature]
Record d by

Date

10-27-84

74

Project No. _____

Book No. _____

TITLE _____

From Pag No. _____

make 2.5 u/l vTag (EKBT1 lot) by 1:1 dilution
of 5 u/l (on P 61) with storage buffer;

vNATag P 61

20 uL

storage buff

20 uL

VP 40 uL

To Page N

Witnessed & Understood by me,

Deborah Powers

Date

11/29/94

Inv nted by

Rec rded by

Date

10-27-94

118

Project No. _____
Book No. _____

TITLE Units - on Loads + Pools -

From Page No. _____

Purpose: What to determine total units on Heparin + Queso + the total units pooled - + determine units / gram from crack sample

1. crude '12000
2. after heat shock '12000
3. Load PET.
4. Load Hep '1000
5. Pool 1 Hep '1000
6. Load Queso '1000
7. Pool 1 (1) Queso '1500
8. Pool 2 (2) Queso '1500

$$(7 \times 3) = 21 \text{ samples -}$$

	SAM	CPM1	
1	'1500	1958.00	118
2	'1500	2486.00	42
3	'1500	3196.00	48
4	'1500	2746.00	49
5	'1500	3998.00	7234
6	'1500	5108.00	23
7	'1500	3000.00	72
8	'1500	4990.00	60
9	'1500	5510.00	33
10	'1500	4888.00	59
11	'1500	7964.00	48
12	'1500	8240.00	
13	'1500	7990.00	
14	'1500	10032.00	
15	'1500	8612.00	
16	'1500	428.00	
17	'1500	78186.00	
18	'1500	78040.00	
19	'1500	79558.00	
20	'1500	22.00	
21	'1500	26.00	

18594-X
SA = 49.7

J MZ
4/5/95

To Page N

Witnessed & Understood by me,

Date

Inventoried by

Date

May Longo

4/5/95

Recorded by

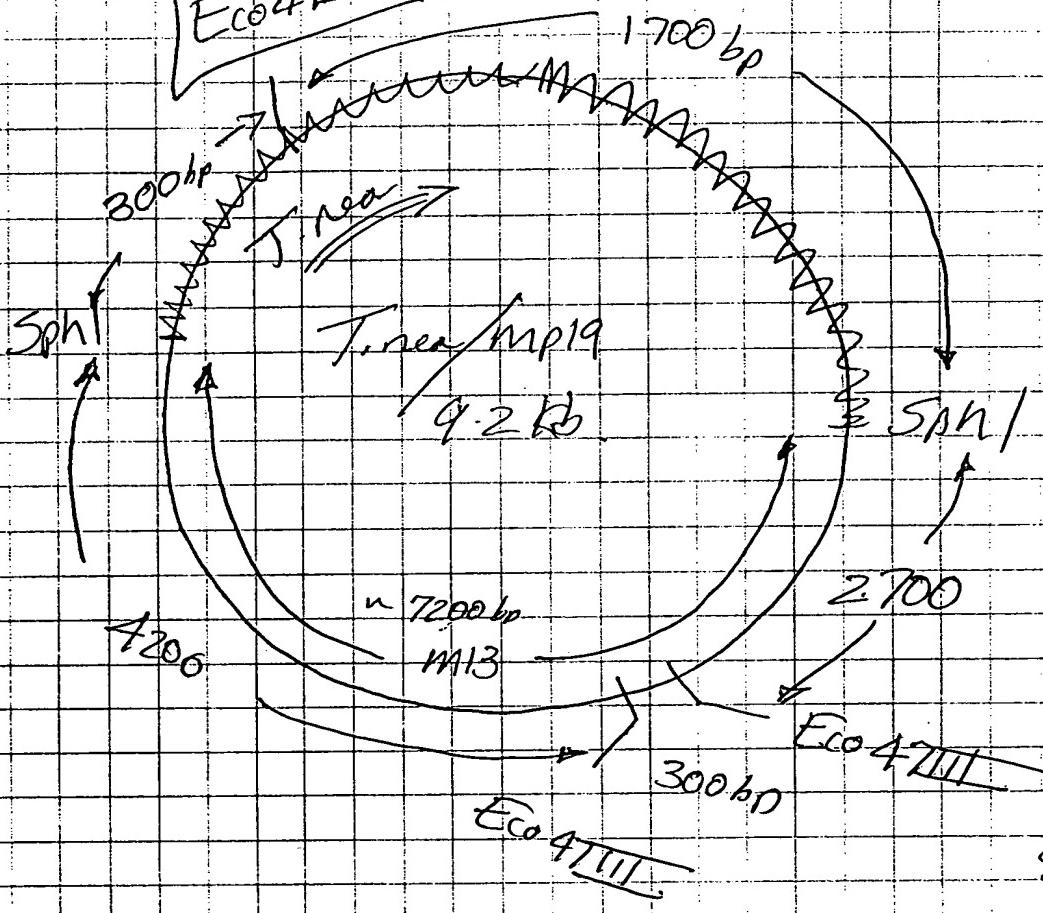
09/04/95

age No. _____

SDM 2899

RF map

2899
SDM
~~Eco 47 III~~



See on file

~~Eco 47 III~~

8.9 Kb

0.3 Kb

1 Kb

5 Kb

4 Kb

3 Kb

2 Kb

1.6 Kb

1.1 Kb

0.5 Kb

0.3 Kb

0.1 Kb

PARENT

MUTANT

PARENT

MUTANT

May be
See light To Page No.

signed & Understood by me,

Date

Invented by

Date

May Jonfo

2/16/95

Recorded by

Douglas J. Schmid

2-16-95

Page No. _____

The samples from previous expt were run on new Tablet agarose from Jiri Spencer. (11/28/94)

1 Tab. = 1 gm

Dissolved 2 gms in 200 ml of 1xTAE (.2 mM EDTA)

Began to dissolve in few minutes at RT in buffer, looked like powder agarose (regular) in buffer.

microwaved for 4-5' (3' didn't completely go into solution)
added 5 ml of 10mg/ml ethidium bromide

easy to pour, no bubbles, like Tengel - looked
not dense, more like regular agarose

when stained looked a bit transparent than regular
agarose, well formed wells.

The gels were run at 100-105 V constant, along with
11x14

DNA mass ladder and Hind III / Lambda.

Looked like it ran a bit faster than regular agarose.

Ladders resolved quite well, the intensity of bands
in mass ladder looked normal.

Apart from convenience of no need to ~~reweigh out~~,
there is no other added advantage.

may be this gel is slightly faster than regular agarose
do it really 1.9%?

To Page No. _____

Read & Understood by me,

Date

12/1/94

Invent'd by

Recorded by

K. S. Iyer

Date

11/29/94

5'P primers for Vent digestion
and 3'5' and/or ribo ends

Same as
P12816

ag N

mer 0.66 μM
(5 μg/l)

VVV 8.1

23 min. Took in
μm 0.66 μM/l

7.1 14.6 μM
= 1 μM

6.1

--

7.2 10.9 μM
= 1 μM

6.1

--

7.9 = 1 μM
= 1 μM

6.1

S -

7.6 38.6 μM
= 1 μM

7.7 59.36 μM
= 1 μM

7.8 11.67 μM
= 1 μM

7.9 6.1 ✓ S

Kinase buffer ✓✓ ✓ 4

32P/ATP 3400 Ci/mmol ✓ 4

mg/ml 11-4-94 ref

PK

H₂O ✓✓ ✓ 1

r_f = 2.0 ml

37°C 30 min

55°C, 5'

Z

✓

Z

✓

Z

✓

Z

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To Pag No.

Signed & Understood by me,

Barbara Polcyn

Date

11/29/94

Inv nted by

Recorded by

Dat

11-1-94

Sample No.	CPM1
1	4148.00
2	4852.00
3	6730.00
4	2580.00
5	3952.00
6	5700.00
7	5318.00
8	3176.00
9	2294.00
10	3002.00
11	8568.00
12	5524.00
13	1742.00
14	1812.00
15	4872.00
16	6352.00
17	242.00
18	82428.00
19	81076.00
20	77332.00
21	

SAM	CPM1	Load
1	4148.00	93 Hepatin
2	4852.00	55
3	6730.00	40
4	2580.00	42
5	3952.00	34
6	5700.00	25
7	5318.00	31.7
8	3176.00	38
9	2294.00	55
10	3002.00	34
11	8568.00	4200
12	5524.00	33
13	1742.00	
14	1812.00	
15	4872.00	
16	6352.00	
17	242.00	
18	82428.00	
19	81076.00	
20	77332.00	
21		

5.80/μl 4.75 positive control
 $\bar{x} = 80278$
 $SA = 50 \text{ cpm/nmol}$

Pooled together the two pools from Q450 - 16.5 ml added .5% w/v

of Triton X + NP-40 dd CTP

Adams premix + 1.1 μl ~~water~~ / 5

48 μl of 3 premix

added to prelabelled

ependants 1, 2, 4 μl

of diluted sample

was added - incubated

for 10 minutes at

74°C - the rxn was

quenched w/ 10 μl

9.5 M EDTA +

ice -

30 μl was spotted on

5 FIC filters -

TCA wash + EtOH wash

dried & counted

aliquots made in serial

$$\left(\frac{10}{100} \right) \left(\frac{1}{x} \right)$$

$$x = 200, 150, 100,$$

	U/μl	Total Units	vol.	mg/ml	total mg	SA	% yield
AS1	6.2 U/μl	1.3×10^4		1.4	3.0		
load		1.3×10^4	21 mL	1.4	3.0	4.3×10^4	
Pool	39.0 U/μl	1.07×10^6	27.5				77.1 new
ad	39.0 U/μl	9.675×10^5	25	.323	8.0	1.22×10^5	3.1 pur.
O/I							
alys	38 U/μl	6.27×10^5	16.5				651 new

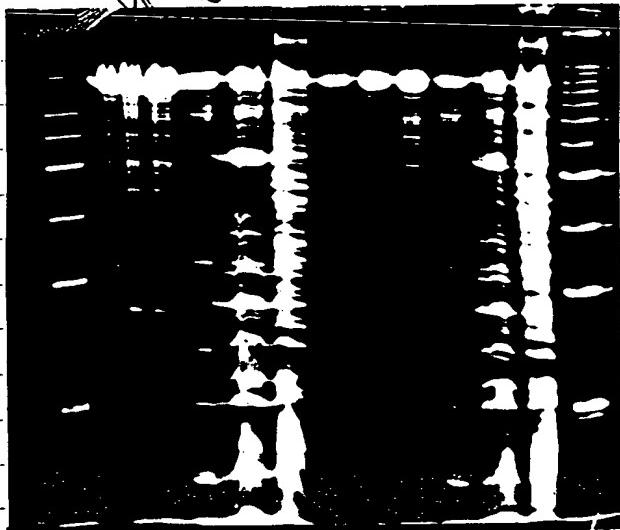
Very conservative ~ 20,250 U/gram cell - for 10 e6 units - 500 gram pack

To Page No. _____

ssed & Understood by me,	Date	Invented by	Recorded by	Date
May Longo	4/5/95	Sig. - May Longo		04/05

Project No. _____
Book No. _____TITLE 6d of Pods - 12.5V. PH6E

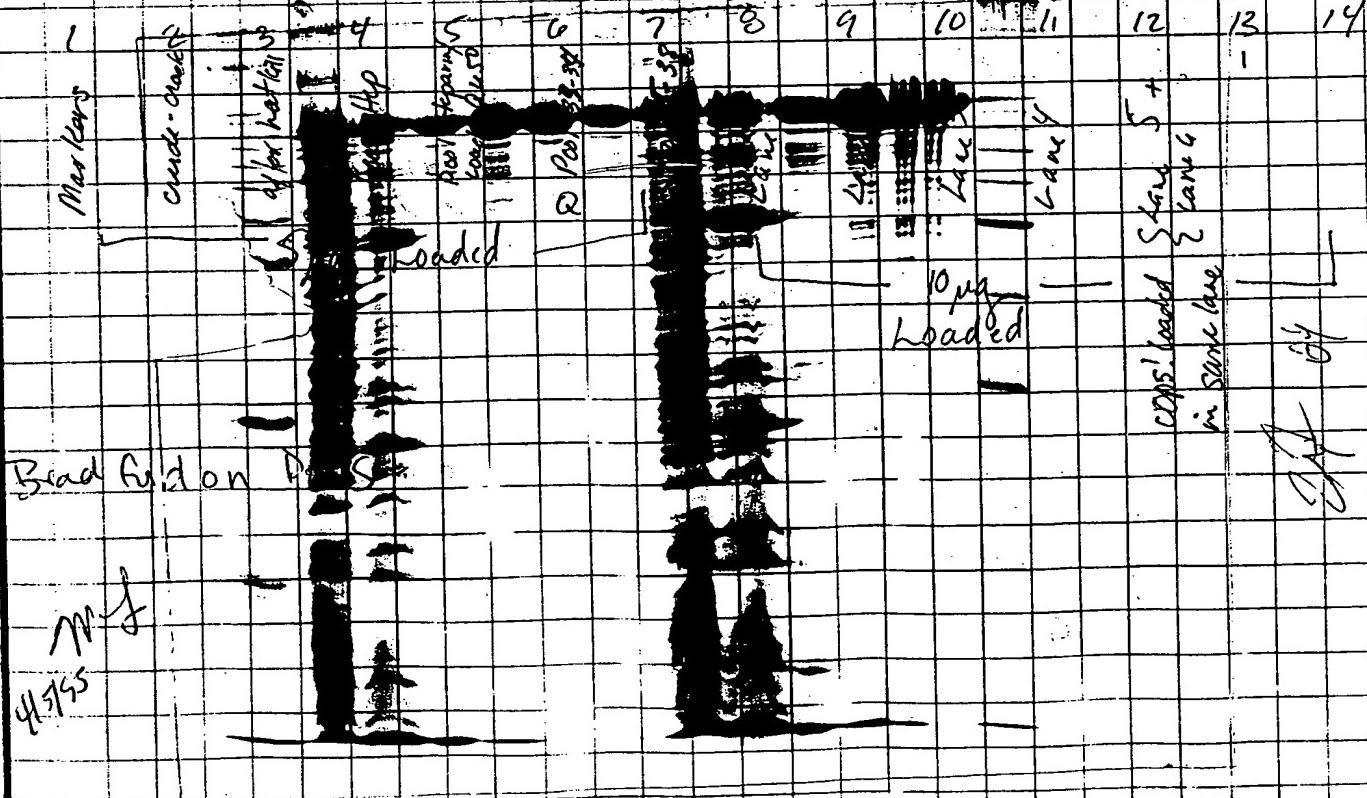
From Page No. _____

13 12 11 10 9 8 7 6 5 4 3 24/5/85

LIFE TECHNOLOGIES, INC.

MK
4/5/85

PH 4/5/85



To Page _____

Witness d & Underst d by me,

Date

Invent d by

E. J. Flynn

Dat

04/05/85

May Forgo

4/5/85

R cored by

QC. RNase Assay -

Page No. _____

Tube	Rxn Mix	Enzyme Unit	µl H ₂ O
1	50 µl	2	4 µl 8.5 U/µl
2		5	1 µl
3		10	2 µl
4		15	3 µl
5		20	4 µl
6		0	-
7	↓	0	-
		Dilute Enzyme - 1/5	
		190 µl True	190 µl
	100 190	950 total volume	(950-190) 760 µl diln Buffer
	dilute to 5 U/µL	= $\frac{1}{7.6} = \frac{10}{7.6} = \frac{10}{(7.6-10)} = \frac{10}{6.6}$ enzyme diln B.C.	

Rxn mix

Tag primers

PCR mix 10x Green AG 8 µl

mRNA Glorin
processed H₂O160 µl + 320 µl 8 µl
232 µl 72 µl
+ 8 µl

400 µl

Incubate at 37°C in heat block
for 1 hour -Add 7 µg Proteinase K (12 mg/ml) + 3 2nd
25 µg t-RNA (5 mg/ml) 2.5rd

Incubate 10 min @ 37°C

Add 20 µl 2M NaAc + 200 µl 100% EtOH - unfix
Keep in Freezer - 20°C 0/V

195.

To Page No. _____

Signed & Understood by me,

Date

Invented by

May Tongo

4/5/95

Date

Elizabeth Flynn

Recorded by

4/5/95

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

2/15/95 wed.

(+) Strand (ssDNA) lot # ED5702 260 μg/ml
RF Strand (dsDNA) lot # CC3111 5 μg/18.4 μl

calculation: ssDNA = 260 μg/ml = ng/μl

$$\frac{260 \text{ } \mu\text{g/ml}}{1000 \text{ } \text{ng/}\mu\text{l}} = 1000 \text{ } \text{ng/}\mu\text{g} \text{ ml} \cdot 1000 \text{ } \mu\text{l}$$

$$= 0.260 \text{ } \mu\text{g/}\mu\text{l.}$$

$$1000 \text{ } \text{ng/}\mu\text{g} (-.260 \text{ } \mu\text{g}) = 260 \text{ } \text{ng/}\mu\text{l}$$

$$\frac{260 \text{ } \text{ng/}\mu\text{l}}{x \text{ } \mu\text{l}} = 100 \text{ } \text{ng}$$

$$\left\{ \begin{array}{l} 260 \left(\frac{1}{2.6} \right) = 100 \text{ } \text{ng} \\ \text{or} \\ \frac{260}{2.6} = 100 \text{ } \text{ng.} \end{array} \right.$$

for 2.6 total or final Volume
you need 1.0 μl DNA

$$\therefore 1 \text{ } \mu\text{l DNA (260 } \mu\text{g/}\mu\text{l)} \\ \frac{1.6 \text{ } \mu\text{l TE}}{2.6 \text{ } \mu\text{l}}$$

for 100 ng/μl, { 2.0 μl DNA (260 ng/μl)
multiply by 2 3.2 μl TE

$$\text{dsDNA} = 5 \text{ } \mu\text{g/18.4 } \mu\text{l.}$$

$$1000 \text{ } \text{ng/}\mu\text{g} \times 5 \text{ } \mu\text{g} = \frac{1000 \text{ } \text{ng}(5 \text{ } \mu\text{g})}{18.4 \text{ } \mu\text{l}} = 5000 \text{ } \text{ng/18.4 } \mu\text{l}$$

$$\frac{5000 \text{ } \text{ng}}{18.4 \text{ } \mu\text{l}} = \frac{272 \text{ } \text{ng/}\mu\text{l}}{x \text{ } \mu\text{l}} = 2.72 \text{ } \text{ng.}$$

for 2.7 total volume you need
1.0 μl DNA

Total Volume (TV)

To Pag 1

With ss d & Understood by me,



Date

4/12/95

Invent d by

R c rded by 

Dat

4/12/95

ag No.	RF (ds)	Tube #1	Tube #2
DNA	1.0 μl × 3	= 3.0 μl	DNA = 1.0 μl × 3 = 3.0 μl
TE	1.7 μl × 3	= 5.1 μl	TE = 1.6 μl × 3 = 4.8 μl
TV	2.7 μl × 3	= 8.1 μl	TV = 2.6 μl × 3 = 7.8 μl

Tube # 1, 2, 3, 4 of RF (ds DNA)

(1)

(2)

(3)

(4)

Alu II

Hind III

Sau 3 A I

Bam HI

H₂O

16.0 μl

10x Buffer

2.0 μl

(React 1; React 2; React 4; React 3)

DNA

1.0 μl

Call 4 tubes w/ 16.0 μl

Alu II

+

-

-

-

Hind III

-

+

-

-

Sau 3 A I

-

-

+

-

Bam HI

+

-

-

+

Tube # 1, 2, 3, 4 of + (ssDNA) same order as RF

2 tubes were set-up for uncut, 1 with RF & 1 with +

- each tube added 16.0 μl H₂O

- 2.0 μl React 2 10x buffer

- 1.0 μl DNA

- Put all 10 tubes in

- ran the sample (all 10) on a gel next morning.
(0.8% agarose gel, 147 volts)

- picture of the gel is on the next pg (pg # 8)

To Page No. _____

ssed & Understood by me,

Dolores

Date

4/12/95

Invent d by

Record d by *Dolan*

Date

4/12/95

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

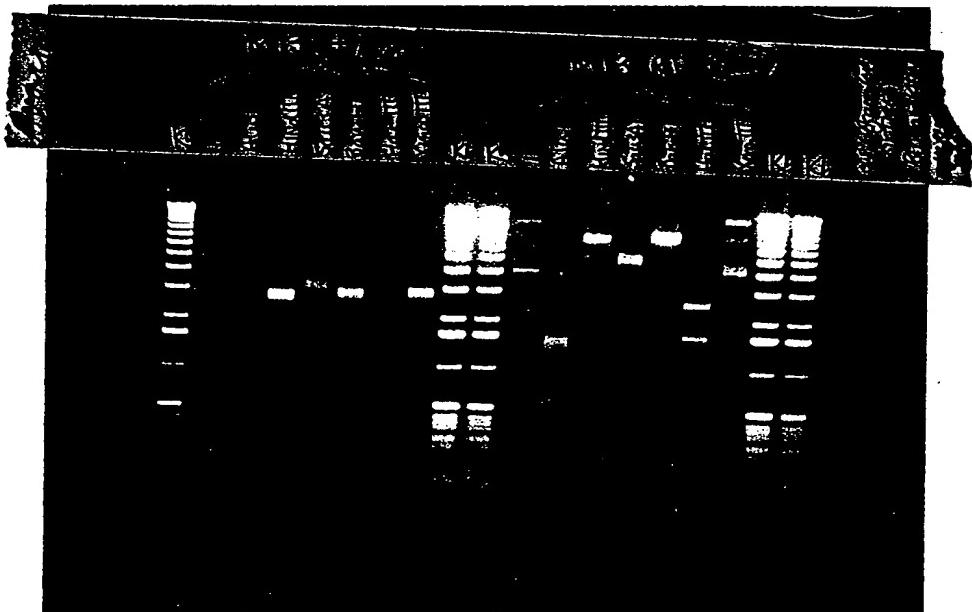
Tube #1 T-nea / pTTC

1.0 ml

Tube #2 T-nea / pTTG

1.0 ml

- Cfg. for 1 min. at room temperature
- discarded supernate and added: 100 μ l SI to the pellet. mixed
- 200 μ l S2 lysis put both tub ice.
- 150 μ l S2 with RNASE A
- Cfg for 5 min. at 4°C
- transferred 400 μ l of supernatant to the new tubes.
- added 800.0 μ l ETOH to the supernatant
- put both tubes in the fridge till tomorrow (2/16/95)



arp 4/12/95

(+) Sal3A1 - gel shift (did not cut
but binded)

To Page No. _____

Witnessed & Understood by me,

Dat

4/12/95

Inv nt d by

Recorded by

Dat

4/12/95

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Appl. No. 09/558,421

Project No. _____
112 Book No. _____ TITLE _____

From Page No. _____

Purpose: Since results are inconclusive - dV (new) (control) gave more specific smear with dependent - previous & (page 110) and gave the same type of smear with old + dV specimen attempted to see whether this smear can be transformed into bands!

altered few conditions checked a diff amount of sample
↓

according to NCB suggestions: 200 & 1000 pg

1. reduce amount of Tempolymer tried first 3.
2. " " & of cycles
3. increase Mg 20 & 30 cycles
4. " " dNTP 2, 4 & 6 mM.

= also included as controls were: Tag + (Tag + D.V.)

tried at 200 pg, 30 cycles, 200 pm dNTP with 2, 4, 6 mM

Two sets of reactions were made one with old dV &
and the other with new - dV specimens.

Added cocktail with different enzymes + later added
more Mg accordingly. Used 10x dependent buffer
has 2 mM Mg already.

- except for the Tag + (Tag + DV) controls rest were non
duplicable.

used 1:0.01 mix 94°, 3'
20 or (94°, 30"
30 (58°, 30"
72°, 3')

Dry Vent used 1 Mod. 1 machine

DV/x

To Page 1

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Date

Invented by

Date

[Signature]

12/10/94

Recorded by
[Signature]

11/30/94

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Project No. _____

Book No. _____

113

age No. ~~new~~ new du premier Ant II

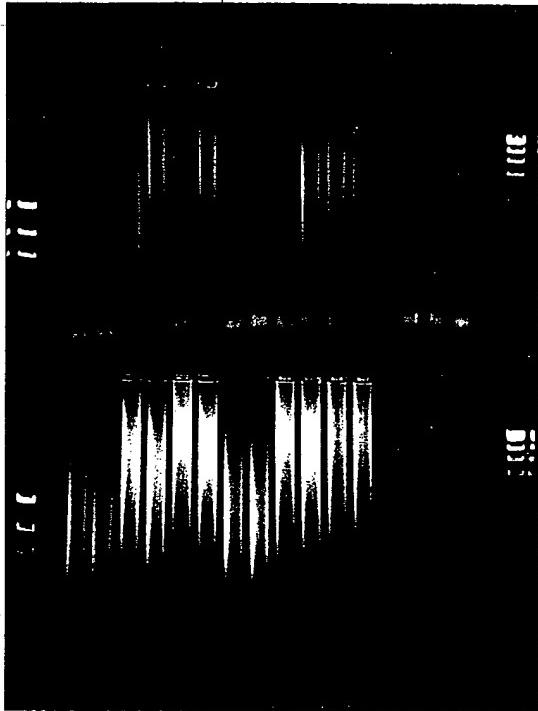
~~old~~ old premier 2728 & 2729

Deep Vent

Tag

Deep Vent

Tag
642



2s
cy

30
cy

Pg 200

100

642 mm ray

2 4 1 2 4 6 Tag + DV

200

100

✓

With Deep Vent
these premieres
give smudges

nothing worked

Cassiniant
- With Tag + DV
works
w. Tag alone
it doesn't

With Tag alone and
Tag + Deep Vent
nothing real clear
smudges / results
reproduces

at 35° azimuth

New premieres are no good.

To Page No. _____

Signed & Understood by me,

Date

12/10/84

Invented by

Recorded by

R. Sankaran

Date

11/30/84

Project No. _____

Book No. _____

Digestion of 5⁴ 23mer by Ven
TITLE ~~ETFL~~, Cheng vs Ven buffer.

76

From Page No. _____

(1) (2) (3) (4) (5) (6) (7) (8) (9)

also
with

rebo a
fS en

3P 23 mer (P75) 3 3 3 3

2P 2791 3

P 2692

4P 2696

P 2698

10P 2700

10X Ven buffer

10 10

3

3

3

3

5X GTM Cheng

20 20

10

X ✓

Mg(OAc)₂ 13 mM

7.5 7.1

X

0.9 ml
+ 0.3 ml
(F:1.2 : 1)

Vent 0.1 uL

1

X ✓

TFI 1 uL

1.24

1.24

X ✓

A1.0

86

75

68.5

70

86

X ✓

if = 100 μ L

70 °C

remove 10 μ L to 5 μ L cycle seq. stop solution at

2, 5, 10, 20, 60 for (1)-(4)

(well 1 is 23mer O time)

and

0, 3, 10, 60 for (5)-(9)

(take O point before pul add)

Program 143 = 70 °C

To Page No. _____

Witnessed & Understood by me,

Deanne A Polkay

Date

11/29/94

Invented by

Recorded by

Date

11.2.94

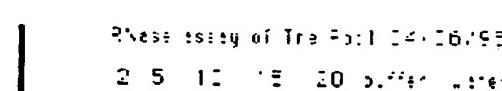
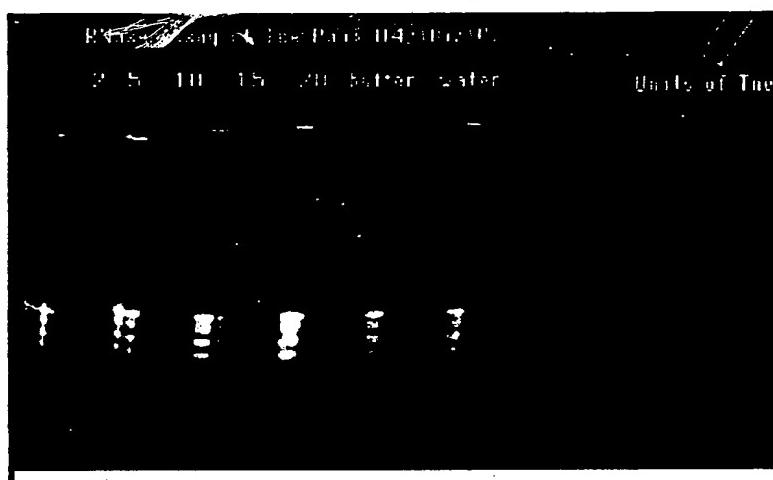
22

Project No. _____
Book No. _____

0
j.m.w.
TITLE Completion of RNase Assay -

From Page No. _____

Take samples from -20°C freezer - spin in micro centrifuge
15 minutes -
decant ETOH - air dry pellets -
Add 1 ml of RNA blue juice - heat 30 sec at 90°C
Run out on 16%
Sequencing gel -
400 volts -



Conclusion -
Appears to be
RNase free! Next
time use more ETOH -
Only used half of
recommended amount
Used 1ug v- recom 2ug.

Bradford on Pools

LIFE TECHNOLOGIES, INC.

0
j.m.w.
4/13/95

To Page N

Witnessed & Understood by me,

May Longo

Date.

4/13/95

Invented by

E. Flynn

Recorded by

Date

04/06/95

Exonuclease Assay - The Pool

Lab No. _____

Cat. No.: 30042 SOP -

tube	Rxn mix 45	Enzyme Units μ	H_2O
1		0.0	-
2		1.2-2.0	5 μ l
3		2.5	4 μ l - 50 μ l
4		1.0	2
5		1.5	3
6		2.0	4
7		0	5 μ l dil'n buffer

Rxn mix
16 rxns -

10x PCR 80

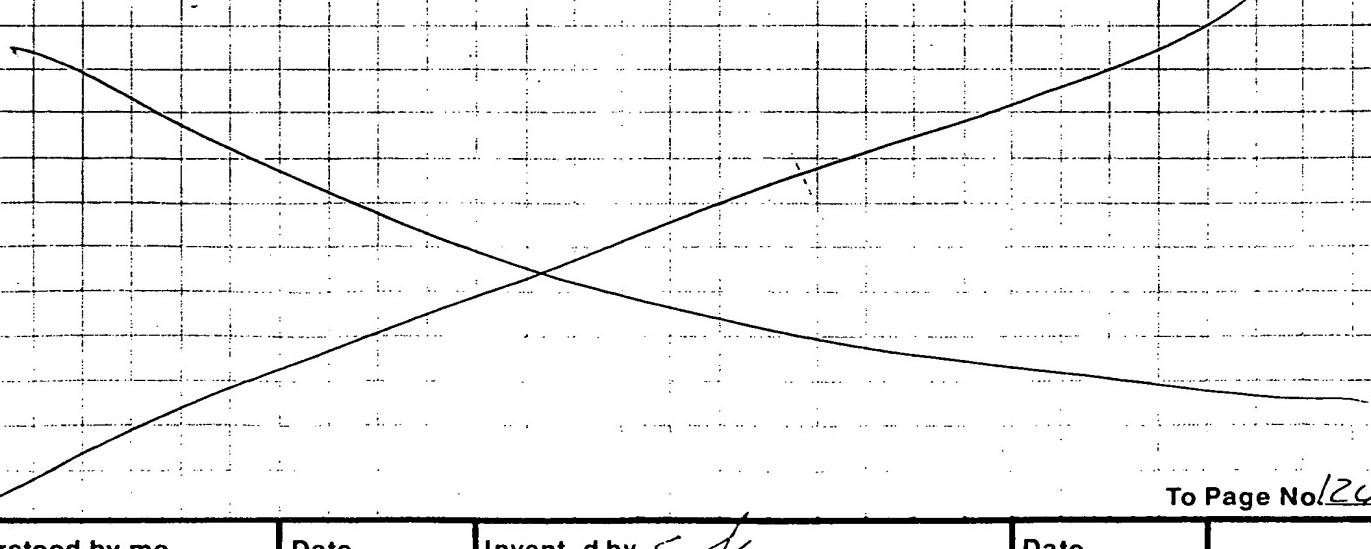
50mM MgCl₂ 80

5' ds sub 16 pmol 32 μ l .5 pmol/ μ l

3' ds sub 16 pmol 32 μ l .5 pmol/ μ l

H₂O 494

720

heat \square 37°C for 1 hour - 1-7heat \square 72°C for 1 hour - 8-14see page - 126
for data

To Page No 126

Issued & Understood by me,

May Tong

Date

4/13/95

Invent'd by

E. Flynn

Date

04/13/95

Rec'd by

Project No.

Book No.

TITLE Endo Assay - 18038 QCP-T

From Page No. _____

Rxn Mixture - in 8 rxns -

(all tubes twice
before use -)

10x PCR buffer - 40 μ l
 50mM MgCl₂ - 40 μ l
 ϕ X174 (+) DNA - 8 μ g (23.5 μ L) ✓
 15264-0B Autoclaved H₂O 25L. 5
 .33 μ g/ μ L 360 μ l

Endo mix

360 μ lH₂O

5

Diluted enzyme 50/ μ l

1	45
2	45
3	45
4	45
5	45
6	45
7	45

2 units	- 2 μ l
5 units	- 1 μ l
10 units	2 μ l
15 units	3 μ l
20 units	4 μ l

5 Diln Buffer ✓

Incubate at 37°C for 3 hours -

37°C

5.5 hours

Tag

Double Strand Assay -

10x PCR buffer 40 ✓
 50mM MgCl₂ 40 ✓
 25260-027 - ϕ X174 RF 8 PCR 24.2 ✓
 EF 1702 Autoclaved H₂O 25L. 8
 .33 μ g/ μ L 360 -

Endo

H₂O

360 -

Dil. Enzyme 50/ μ l

1	45
2	45
3	45
4	45
5	45
6	45
7	45

H₂O

5

2 μ l	.50/ μ l
5	1 μ l
10	2 μ l
15	3 μ l
20	4 μ l

5 Diln Buffer ✓

To Pag 1

Witnessed & Understood by me,

Date

Invented by

Eliz. B. Flynn

Dat

04-04-95

Mary Fongo

4/13/95

Recorded by

Endo Assay

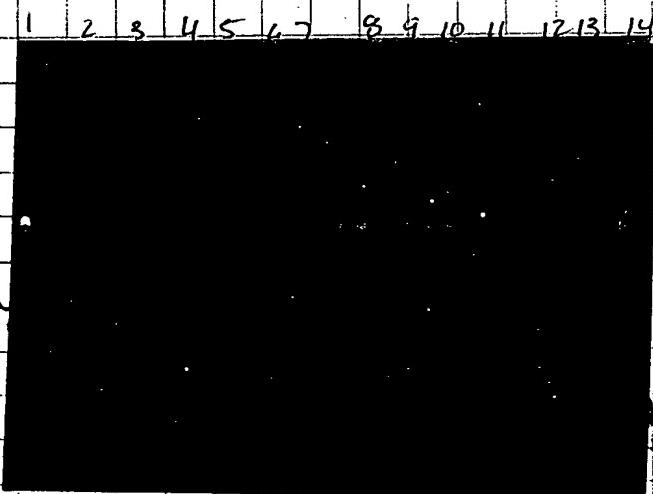
Project No. _____

Book No. _____

125

Page N. _____

Spin samples down and add 5ul of Blue Trice -
run out on 1.2% Agarose gel -



Single Endo DS-Endo

Add

1	2	3	4	5	6	7	8	9	10
H ₂ O	2	5	10	15	20	B			

8	9	10	11	12	13	14
H ₂ O	2	5	10	15	20	B

CONV

C = 100	at 10u - 45	- 3.11/3
100	10	10.11/3
	45	

11335

Endo looks good - however DS Endo - shows conversion to linear but this is also present in the buffer only lane - 1, could just be a contaminant in the Dil'n Buffer -

Dil'n Buffer used - from A.G. flakers from the 4°C Deep cooler - orange tip -

Inclusion: - free of SS Endo nuclease - possible DS endo nuclease but control wth buffer only shows significant conversion to linear so believe this is the dil'n Buffer it also has DS^{endo} activity. mat he change prep.

some

To Page No. _____

Assessed & Understood by m ,

Date

Initiated by

S. Flynn

Date

55/04/95

Mary Tongo

4/13/95

Recorded by

Tne mutant Phe to AlaProject N _____
Book N _____

ge No. _____

- (1) The same phenyl alanine corresponding to Tag polymerase will be changed to tyrosine.
- (2) For exo D will be changed to Alanine (corresponding region of Tag).

Brian cloned the Sphi I fragment of Tne Pol into M13mp.

I isolated the single stranded DNA from CJ236 as described before in Bio rad manual.

Test 5 μl ssDNA



The DNA looks real good.

For D-A (3'-5' exo mutant oligo) is

~~G A | C G T | T T C | A A G | C G C | T A G | G G C | A A A | A G A~~ # 2899
EcoRI site

For Phe → Tyr (O-helix) HpaI.

~~G T A | T A T | T A T | A G A | G T A | G T T | A A C | C A T | C T C | T C C | A~~ # 2904

ian kinased 2899 before.

kinased 2904 as follows:

2 μl oligo (210 pmol)

6 μl 5X buffer (350mM Tris pH 7.6, 50mM MgCl₂, 50mM KCl, 5mM PME)

1 μl 10mM ATP

0.5 μl T4 Kinase (5U)

20.5 μl H₂O

5° at 37° → ~~Heat at 65° + 30 min~~
To Page 2

signed & Understood by me,

Date

4/1/95

Invented by

Recorded by

Date

3/14/95

Project No. _____

Book No. _____

TITLE _____

114

11/30/94

From Page No. _____

Purpose: Amplify pGAPDH for analysis - cloning and transformation w/ different enzymes again.

- GAPDH / Regular Forward & reverse primers / Deep Vent worked (pg. 97)
- never tried with Tag + Deep Vent,
- but no problem with Tag alone (pg. 88).

95° 5°

need: Deep Vent buffer - 2 mM Mg²⁺ (94°, 30", 60°, 1' 15")
200 μM dNTP
0.5 μM primer
100 μg template (10/μg/λ)

72°, 10'

Tag	units	Tag + Deep Vent				Deep Vent	
		31	32	10 + .001	.005	15	16
1	2	0	33	34			
3	4	.5	35	36	.01	17	18
5	6	1	37	38	.05	19	20
7	8	1.5	39	40	.1	21	22
9	10	2	41	42	.2	23	24
11	12	2.5	43	44	.5	25	26
13	14	5	29	30	1	27	28
15			17 X			45	46
	15X					47	48

10x buffer	75	Cocktail →	50 μl/Rx	20X
dNTP	15			1
Template	150		+ 50 μl,	
primer 1	3.75		+ 1 μl	
2	3.75		+ 1 μl Tag in all	di f. am 2
				dep Vent

added enzyme
separately in 1 μl

To Page

Witnessed & Und rstood by me,

Date/

12/14/94

Invented by

Recorded by

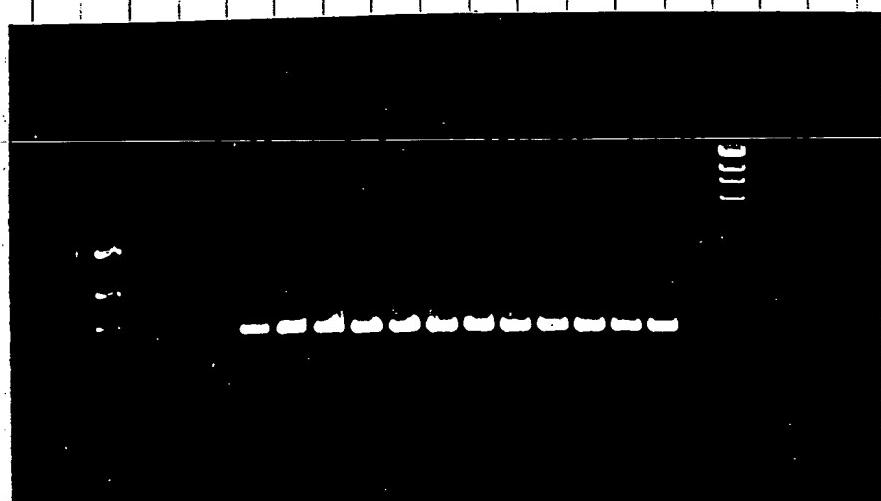
Date

11/30/94

R. Shaneman

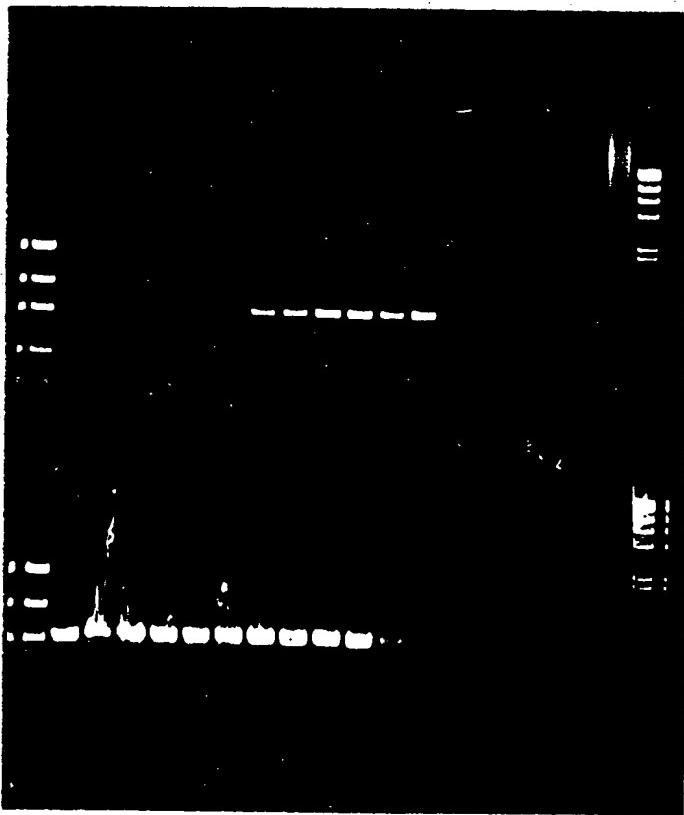
tag No._____

tag



c .5 1 1.5 2 2.5 5

.001 .005 .01 .05 .1 .2 .5 1 2



"UV tag + 0.01 .005 .05 .1 .2 .5" UV Deepvent

Result:

- 100 pg + 25 cycles seem to be enough
- Deep Vent alone with regular primers worked again
- Deep Vent at lower concentration works better. with 0.05 V good product yield seems seen.
- As may be worth earlier runs, if the enzyme con is lower, it might have worked with Deep Vent. Try?

To Page No. _____

Signed & Understood by me,

Date

12/1/94

Invented by

Date

12/1/94

Recorded by

K. S. Srivaman

Project No. _____

Book N. _____

TITLE _____

78

From Pag No. _____

cycle seq.
one pool

A C T A G T

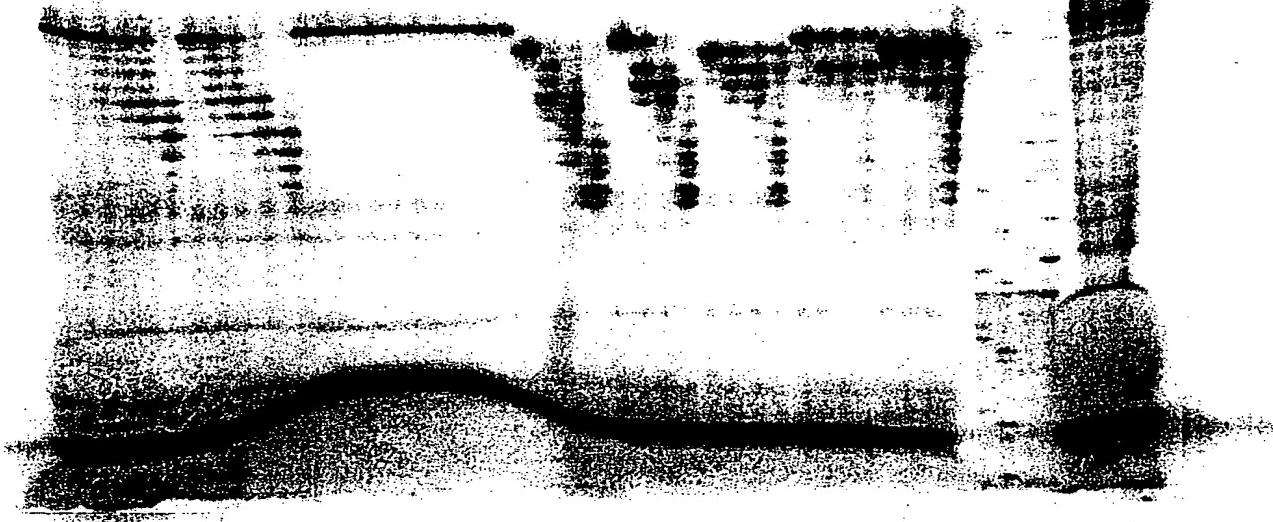
TF

min

Vent buffer, Chevy Buffer

- + - +
025102060 25N2060
25102060 25102060

-- -r S- SR -S
031060 031060 031060 031060 031060



ON Apr 3 211-10000
say,

To Pag _____

Witnessed & Understood by me,

Doreen Stump

Date

11/29/94

Inv nted by

R c rded by

Date

11-2-94

EX-130/75

Page No.

Wall BTR

(ml)

- Success rate 11.5 grams for 30 ml of CEC

50 mM Tris - 7.5 - 100 ml
0.5 mM EDTA - 2 ml
0.2 mM PMSF - 4 ml
Sagittal - 160 ml
5 mM B-ml - 69.9 ml
10 mM KCl - 6.7 ml

inh vol = 40 ml = 0.65 ml/l

1.4 ml 10% PEI

Sandata w/ med. TIP - output - 8 -
3X / min -

1.200 ml after 55

Crude = 0.74

3 = 0.17

- Heat kill 85°C for 10 min

- Cool. 0. Make 50 mM KCl (final conc.)

© ADD 0.4% PEI

→ Spin in 55-54 15 min - 200's -

Decant supernatant

6.0% Ntly 50% cut = 590 g/l = 12.5

→ 1.7 grams/ml resin

Equilibrated 8 ml T850 Hybrane Column w/ 1. - 8 ml = 3.24 x 0.25 =

2) 25 mM Tris - 7.4

8 ml = 0.785 = 10.2 cm

Sagittal -

0.5 mM EDTA

10 mM KCl

5 mM B-ml

0.1 mM PMSF.

Same (f) 1.5 ml KCl.

Resuspended pellet 1.1 ml.

- Dialyze 0.1M - in A - vs. 20 vol's -

- 12 VT gradient = 96 ml's

1.6 ml fraction x 60 fractions

gradient = 1.75 ml/ml.

55 min -

signed & Understood by me,

Date

Invented by

Date

May 31/95

5/31/95

Recorded by

5-2245

To Page N

'ag N. _____

2/21/95 TUE

DIGEST T.nea / pSPORT with SstI & SphI

DIGEST M13 mp 18 E1 M13 mp 18 w/ SstI & SphI

M13 mp 18 RF (0.44 ug/ml) } cut 500.0 ng
M13 mp 19 RF (350.0 ug/ml) }

$$\rightarrow 1000 \text{ ng/ml} \times 0.44 \text{ ug} = 440 \text{ ng}$$

$$\frac{500 \text{ ng}}{440 \text{ ng}} = 1.14 \text{ l}$$

$$\rightarrow 1000 \times 0.350 \text{ ug/ml} = 350 \text{ ng}$$

$$\frac{\cancel{350}}{350} \frac{500 \text{ ng}}{350} = 1.4 \text{ ml}$$

mp 18

H₂O - 35.0 ml
10x buffer - 2.0 ml ← REact 2 → 10x buffer
500ng DNA - 1.1 ml
1ml SstI - 2.0 ml
40.0 ml

mp 19

H₂O - 35.0 ml
2.0 ml
DNA 1.4 ml
SstI 2.0 ml
40.0 ml

T.nea / pSPORT

H₂O - 81.0 ml
10x buffer - 10.0 ml
ng/ml DNA - 4.0 ml
SstI = 5.0 ml
100.0 ml

- Incubated all 3 tubes @ 37°C for 1/2 hour
- Made 0.8% agarose gel
- 250.0 ml TE buffer
- 2.0 g Agarose
- boiled for 4.0 min.
- added 12.0 ml E. Biomide
- poured the gel.

To Page No. _____

signed & Understood by me,

Date

Invented by

Date

4/12/95

Recorded by

4/12/95

Project No. _____

Book No. _____

TITLE _____

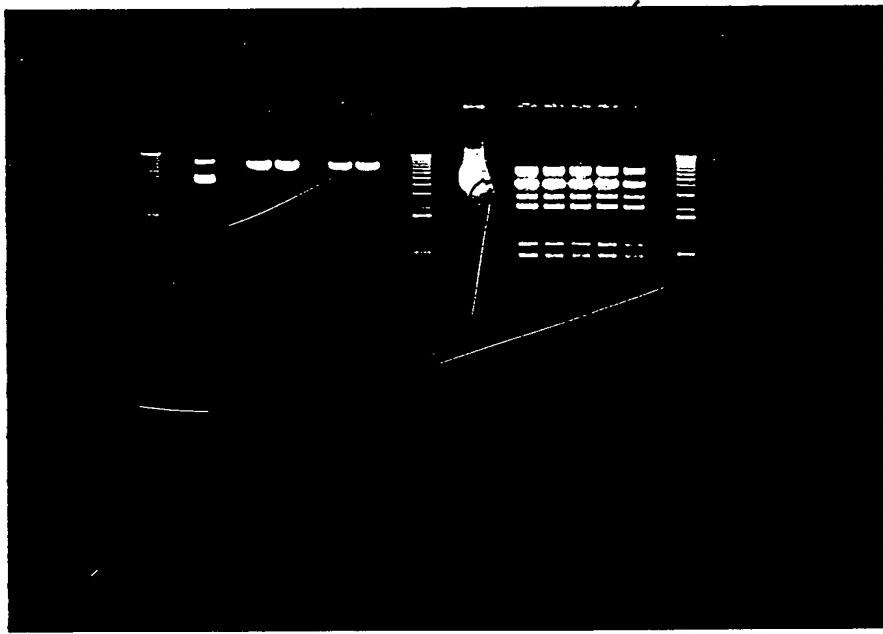
From Page No. _____

added: 2.0 μl of 1M KCl $2 \text{ mg} = 2000 \text{ ng}$
 to 100.0 ng (mp 18 & 19)

5.0 μl of 1M KCl
 to 100.0 ng (pSPORT)

added Sph I - 2.0 μl mp 18
 2.0 μl mp 19
 5.0 μl pSPORT

- Incubated @ 37°C for 1/2 hour
- put the tubes in the fridge till
- ran samples on the gel ~~to~~ on 2/22/95



arp 2/22/95 (2)

M13 mp 18 & mp 19 RF D
 are ds, supercoiled forms
 the DNAs of phages M13
 & 19. Using this vector
 foreign DNA can be
 inserted into the unique
 cloning site in an
 oriented fashion.

Witnessed & Understood by m ,

Date

4/1/95

Invented by

Recorded by

Date

4/12/95

T Pag N

116

12/1/94

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

Purpose : - To try new primers again with PCR
to get rid of mispriming & optimizing choice of Mg.

used: Klenow buffer w/o Mg added Mg later, different co 5 ml.

- 1 Unit Tag		mm	1	1.5	2	2.5
- 1 pm primer included		5	7.5	10	12.5	
- 20 ng template primer 1 alone		25	42.5	40	37.5	3
200 pm dNTP		50				
w/o primer		1				
w/o Mg as controls		5 ml / rx				

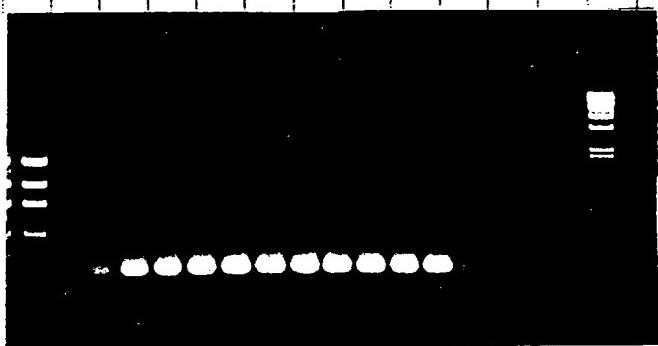
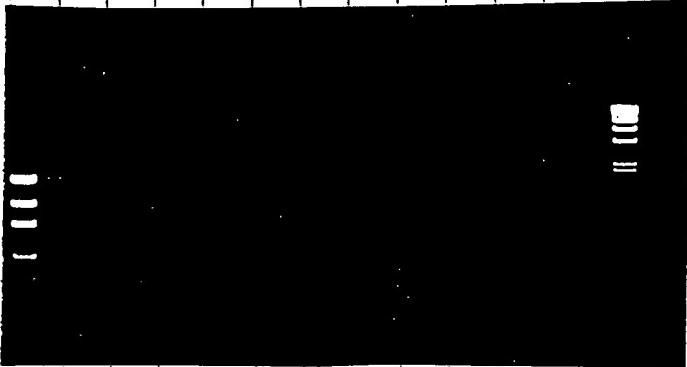
Cycling : 94° 3' 30(94°, 30", 56°, 30", 72°, 3) 72°, 10' 4'.

10 x buffer	60
dNTP	12
Template	2.4
enzymes	2.4
primer 1	6.0
2	6.0
1120	1451.2

- Did the same with new dU primers.

2728	
2729	
1120	
1	Mg.
45 ml + 5 ml	

w/o primers assembled separately.



To Pag

Witnessed & Understood by me,

Date

12/19/84

Invented by

Date

12/2/94

Recorded by

Job No. _____

Result: no product in both sets of reactions.

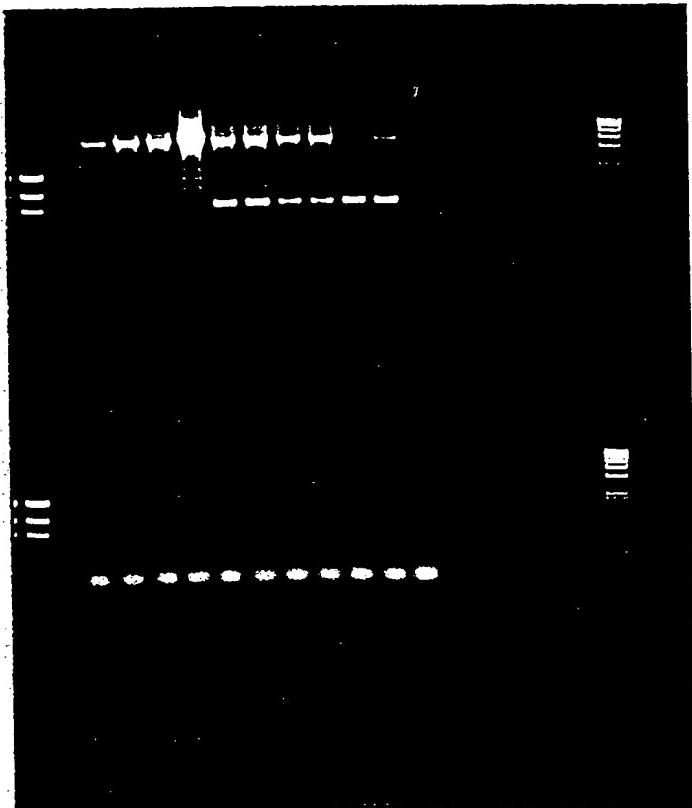
more primer dimer with new dU primers?

1 U of Tag, 200 pM Template for 6.1 kb enzyme might not be enough. And they try 102 - gel products in Klenow buffer but not of this priming was observed.

Since it didn't work & no products were seen don't know about the effect of Mg.

repeated the expt again with dU-primer + Tag (0.61 + 1 U)

dU 1 1.5 2 2.5 3 mM Mg



New dU

Result:

- Once again new dU primer didn't work even with this enzyme cocktail

- Less Mg = less mispriming
the lower band starts to disappear.

- 3 mM less product than 1.5 - 2.5 mM.

- Again with dU-new primer more primer dimer

* * Try with more enzyme
Tag - 2 U

To Page No. _____

Read & Understood by me,

Date

12/14/94

Invented by

Recorded by

J. S. Banerjee

Date

12/14/94

130

Project No. _____
Book No. _____

TITLE

7850 HEP 650 m-
Report of Unit Assay - Enzyme

From Page No. _____

8 ml column -
Cross Columns -

Curve
Post Hb.
Am 503-Sop

10.10

10.15

10.20

10.25

10.30

10.35

10.40

10.45

10.50

10.55

10.60

10.65

10.70

10.75

10.80

10.85

10.90

10.95

11.00

11.05

11.10

11.15

11.20

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11.30

11.35

11.40

11.45

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11.60

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12.00

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15.85

15.90

15.95

16.00

16.05

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17.60

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17.75

17.80

17.85

17.90

17.95

18.00

18.05

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18.30

18.35

18.40

18.45

18.50

18.55

18.60

18.65

18.70

18.75

18.80

18.85

18.90

18.95

19.00

19.05

19.10

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19.45

19.50

19.55

19.60

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19.70

19.75

19.80

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19.90

19.95

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20.70

20.75

20.80

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20.90

20.95

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21.70

21.75

21.80

21.85

21.90

21.95

22.00

22.05

22.10

22.15

22.20

22.25

22.30

22.35

22.40

22.45

22.50

22.55

22.60

22.65

22.70

22.75

22.80

22.85

22.90

22.95

23.00

23.05

23.10

23.15

23.20

23.25

23.30

23.35

23.40

23.45

23.50

23.55

23.60

23.65

23.70

23.75

23.80

23.85

23.90

23.95

age No. _____

- Sonicate 2x dilution H 3 -
 - Fugue Therm 2x dry ice -

- Heat kill 85°C - 10 min -

- Assay pre & post HC treatment

1	104.00
2	150.00
3	104.00
4	128.00
5	9012.00
6	146.00
7	114568.00

Eggs - A =
 25 mm 7.12-7.4
 81 g/mol
 0.4 mol EDTA
 20 mM KCl
 Tris Base

= Same(t) 1.2 ml/cell

To Page No. _____

Signed & Understood by me,

May Tong

Date

5/31/95

Invented by

Recorded by

Date

5-2595

80

Proj. ct No. _____
Book No. _____

TITLE

23mer degradation: V, UV, Inc
buffers: Cheng vs Vent vs. Klenow.

From Pag. No. _____

① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩ ⑪ ⑫

Cheng buffer 5X
10X Klenow buffer *
Vent buffer

20 → 10 →

10 →

2 2 →

Mg OAc 12 mM

9.5 →

1.2 μl →

Mg SO₄ 100 mM

16 →

glycerol 50%

DMSO 100%

³²P 23mer **

3 μl →

Vent pol 0.05 μl

2 2 2 2

2

Deep Vent 0.05 μl/μl

2

2

2

2

Taq 0.5 μl/μl

2

2

2

H2O

6.5 → 81.8 81.8 83.8 85 → 69 →

Vf = 100 μl

Preheat to 70°C, start by addition of DNA pol
remove 10 μl to 5 μl cycle reg stop mix at 10, 20, 30 min
well #1 is 23mer uncut

To Pag

Witnessed & Understood by me,

Deborah Botany

Date

11/29/94

Invented by

R cord d by

Dat

11-X-94

g. No.

(14) (15)

✓
✓
→ ✓

- ✓ ← (note Klenow system relies on Tag storage buffer for glycerol and Tween₂₀/NP40 - for TnT, it is diluted in Tag storage buffer so no supplement is needed for Vent and Deep Vent dilution is in storage buffer (with Triton and 5% glycerol))
- ✓ (1.2 mM Mg²⁺ Cf)
- ✓ (1.2 mM MgSO₄ (f))
- ✓ Cf = 8% glycerol

→ ✓ Cf = 2% DMSO

→ ✓

2

2

✓

} dilute in Vent/Deep Vent storage/dilution buffer (its 0.1% Triton)
(dilute in Tag storage buffer)

8% Cf = 0.002% Triton

will include
2 ml Tag storage
buffer next time
(similar to 7 ml
storage buffer with
0.5% Tween/NP40)

Primer, 0.66 μM 13.5 μl (8.91 pmol)

~~Primer~~ 16.8 μl (25 pmol)

H₂O 24.2 ~~μl~~ μl

5.5 μl
3 μl
0.66 pmol primer

1.66 pmol H₂O 13.5 μl

** for ³²P23mer mix ³²P23mer of PT
plus 16.8 μl cold 5¹³C 23mer plus
24.2 μl H₂O. Vf = 5 μl and specific
activity is reduced ~~× 2~~ × 2

* UV X Klenow is 500 mM Tris HCl pH 7.
160 mM (NH₄)₂SO₄ and no MgSO₄

To Page No. _____

ed & Und rstood by m .

Dat

Inv nt d by

Date

Doreen Bolcun

11/29/94

R cord d by

11-4-94

1g N. _____

2/22/95

1. grow cells overnight (0/N) 10.0 mL

= 9.0 mL (1.0 mL in ea. nine tubes). Each tubes labelled

AH10B

ptrc1T.nea

2/22/95 BJS

L8 + AP100

• Quick freeze all nine tubes in a powdered
dry ice.

GENE CLEAN

2) Did electrophoresis on yesterday's DNA (2/21/95)

M13 mp 18 and M13 mp 19 and pSPORT

b) Took the picture of the gel

c) cut off mp18 fragment, mp19 fragment & pSPORT fragment from the gel & transformed the gel w/ ¹⁰₁₀ NaI into the separate eppendorf tubes.

d) added 700.0 μ l NaI to each tubes. vortexed mp18 & mp19 tubes.

e) incubated both tubes @ 55°C to melt agarose. mixed after incubation

f) added 5.0 μ l Glass Milk to both tubes.

g) incubated both tubes on ice for 5 min.

h) centrifuged both tubes (quick spin)

i) discarded supernate

j) added 500.0 μ l New Wash buffer

k) discarded supernate & again added 500.0 μ l New Wash buffer.
washed both tubes 3 times.

l) added 10.0 μ l dH₂O to the tubes. mixed well by vortexing. 55°C for 2-5 min

m) set up Ligation

Ligation

$$H_2O = 12.0 \mu l$$

$$H_2O = 12.0 \mu l$$

$$5 \times \text{Buffer} = 4.0 \mu l$$

$$5 \times \text{buffer} = 4.0 \mu l$$

$$mp\ 18\ DNA = 2.0 \mu l$$

$$mp\ 19\ DNA = 2.0 \mu l$$

$$(1 \mu l/\mu l)\text{Ligase} = 2.0 \mu l$$

$$\text{Ligase} = 2.0 \mu l$$

$$TV = 20.0 \mu l$$

$$TV = 20.0 \mu l$$

n) Incubated both tubes overnight @ room temperature (cont'd)

To Page No. _____

Read & Understood by me,

JP Palmer

Date

4/12/95

Invented by

Recorded by

Date

4/12/95

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

(con'd)

T. rea/Ptac E₁ pttc

- 1.0 mL of ea.
 - Cfg.
 - discarded supernate
 - added 100.0 μ l SI, mixed well
-
- Incubated on ice for few min.
 - added 200.0 μ l S2 lysis
 - Incubated on ice for few min.
 - added 150.0 μ l S3 w/ RNase A
 - + Cfg. for 7.0 min. @ 4°C

Witnessed & Understood by me,

D. Polcino

Dat

4/12/95

Inv nt d by

R c rded by

Dat

4/12/95

T Pag

118 12/1/94 Project No. _____
Book No. _____

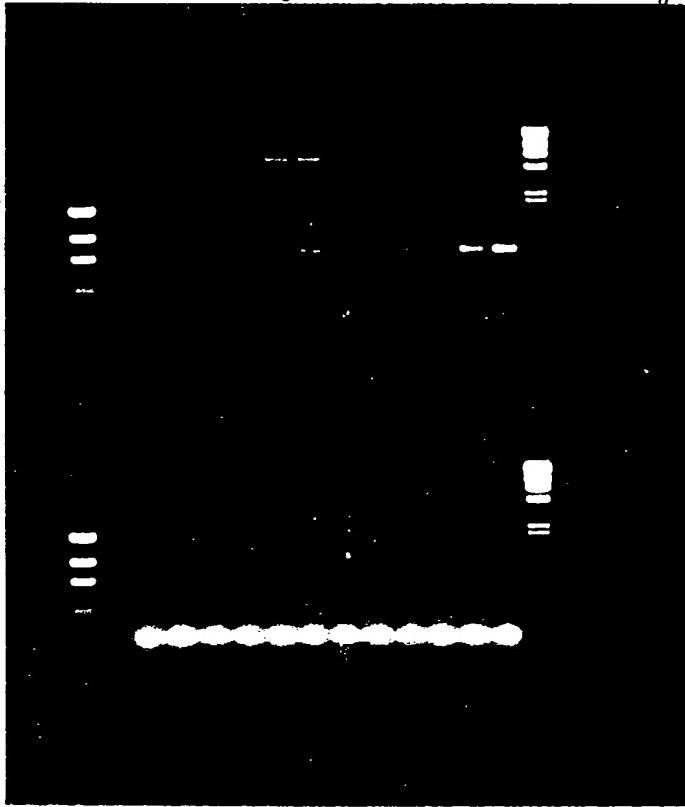
TITLE _____

From Page No. _____

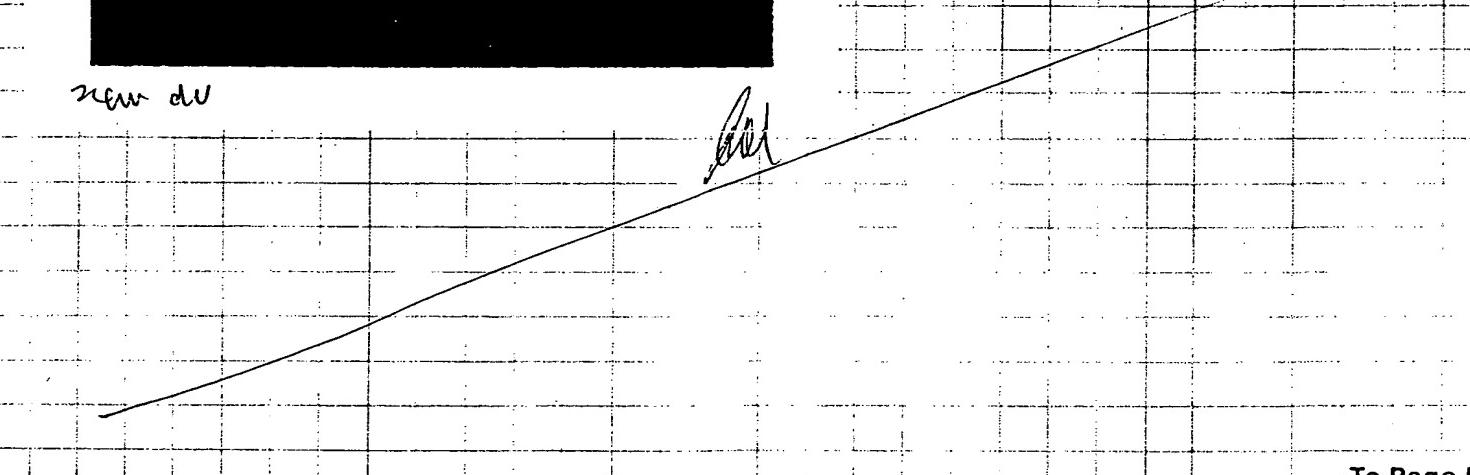
purpose: since 1U of Tag - titration with Mg didn't work, increasing the amount of enzyme to 2U

- Both new + old d_v primers were tried
- Experiment was done under same conditions as I

old d_v 0 1.0 1.5 2.0 2.5 3.0 mM Mg



new d_v



To Page N

Witnessed & Understood by me,

Date

12/1/94

Inv nt d by

Record d by

Date

12/1/94

120

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

- used Max efficiency D45 \times EK3 10 \times to transform
- control pre 5 ml = 50 pg } + 100 µl g Competitor
- 5 µl f each UDG Rx }
- add 900 µl g competitor. cells and let it express for 1 hr.
- plated control - diluted 1:10 20, 50, 100 µl
- Test - un 1, 20, 50 & 100 µl.
- 100 µl from each plated \times 2 12/6/82
- Ayoub counted them all. Amp - depletion resulting in lots of satellite colonies. - he is severe.
- According to Ayoub in Mr treated the counts were in 50 - there not much difference between balanced & ~~injury~~ in 12 reactions, more still more debris than (normal) each time of mine.
- = 10 fold increase in the present set of plates
- = in 10% which.
- Plated a few more from each reaction for better accurate count. These plates were plated with 50 pg of fresh Ampicillin.
- prepared in 6 ml of 100 mg/ml -

To Page 1

Witnessed & Understood by me,

Date 12/6/84

Inv nt d by

Date

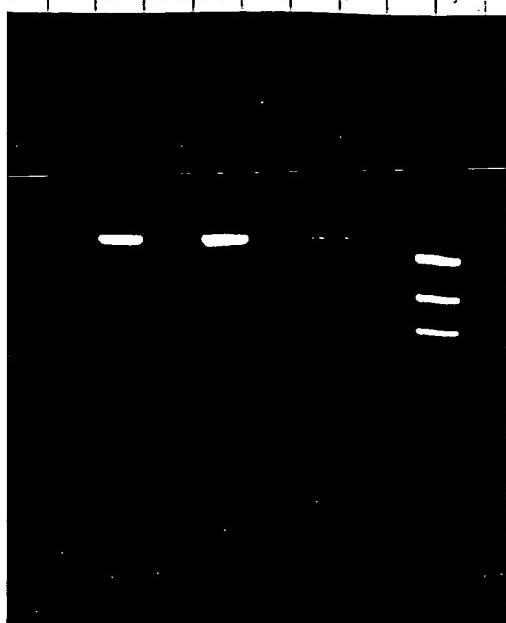
R c rd d by

12/6/84

Ex. - J. L. M. M. A. M.

sg No.)

10 20 50

50 pg
30
20
10
5
2.5

- agents did another simulation with Tag, different cycle #s and 2 diff conc of target completely, 5 pg and 50 pg with dev primers & puc.

- 4 cycles : 10, 20, 35 & 40
with 10 cycles no product in both conc of target

With 20 cycles, 6-pg target barely visible product 50 pg gave a faint amount which was quite weak.

R ₆	R ₇	R ₈	100μl : 6.7 - 4.8 } 44.3
R ₅			" : 5.1 - 4.8 } -
T			5.0 : 4.7 - 2.9 - 3.8 }
Mg	Max		2.0 : 3.1 - 1.4 - 3.2 }
			50 9.0

With 5 & 50 pg, quite a good amount, decent yield with 35 & 40 cycles. With 50 pg more than 5 pg & 40 cycles more than 35 cycles as expected.

Regular c T - 20μl : 3.6 2.7 = 8% { 20 : 9.8 1.1 } = 8.6% { 50 : 2.28 2.0 } = 8.6% ~ 10%

Did transformation of all the products. more amp lysed out 500 μl final.

all with Tag.

Result: colony & too few even at 35 & 40 cycles. But this % of colonies unexpectedly high even at lower & 8 cycles??

op.: no problem - even in BPP plates satellite colonies with 2x S. mut with control something to do with test construct transformation without yield did not correspond w/ % colonies obtaining?
correlation between % of cycles and % of colonies?.

To Page N .

Signed & Understood by me,

Date

12/6/94

Invent d by

Dat

12/7/94

Recorded by

Dr. S. Ishaamow

Project No. _____

Book No. _____

TITLE _____

Exhibit 13

Appl. No. 09/558,421

From Page No. _____

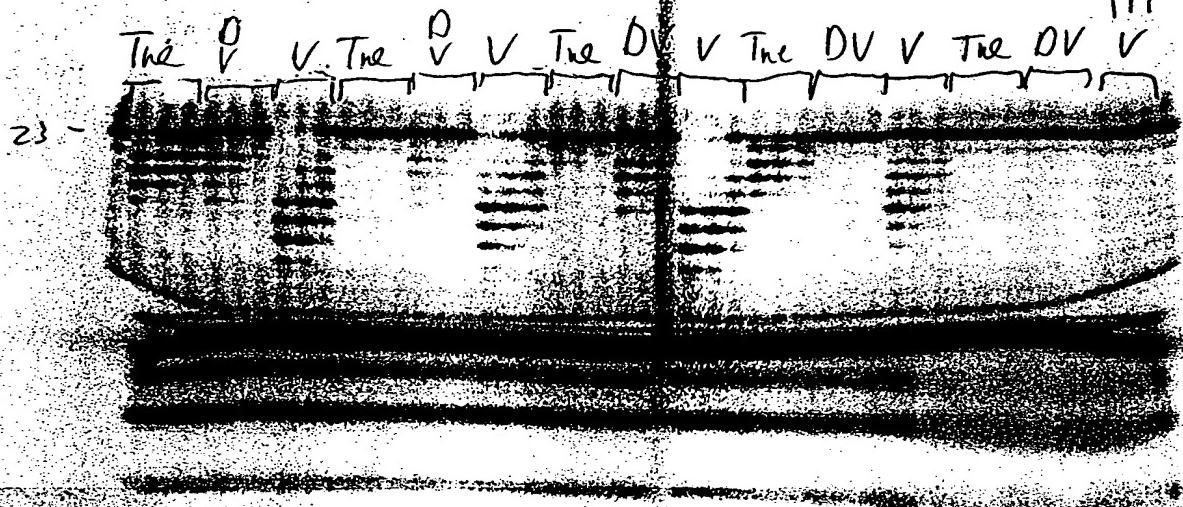
2% DMSO, 8% glycerol

Kleintag
bufferCheng
buffer

302010 min

115

-23



Result.

Witnessed & Understood by me,

Deborah Polans

Date

11/29/94

Invented by

Recorded by

Date

11/5/94

To Page

Project No. _____
Book No. _____ TITLE _____

From Page No. __

	Chen	Kleintag	Vonett
DMSO	20 mM	5-6 mM	20 mM
Tris-HCl pH	9.1	pH	9.1
KCl	25	10	20 mM
$(NH_4)_2SO_4$	1.6	1.0	
MgSO ₄	1.02		
DMSO	2	2	
Triton			
Tween 20/NP40			
Sugammadex	0.01% from tag	1	
Cone Shutter	1.05	1.2	5.0
Vent	+	++	
Deep vent	-	++	
The	-	+	-
			(+ for DMSO)

Witnessed & Understood by me,

Deerara Polay

Dat
"həq̥laq̥

Invented by

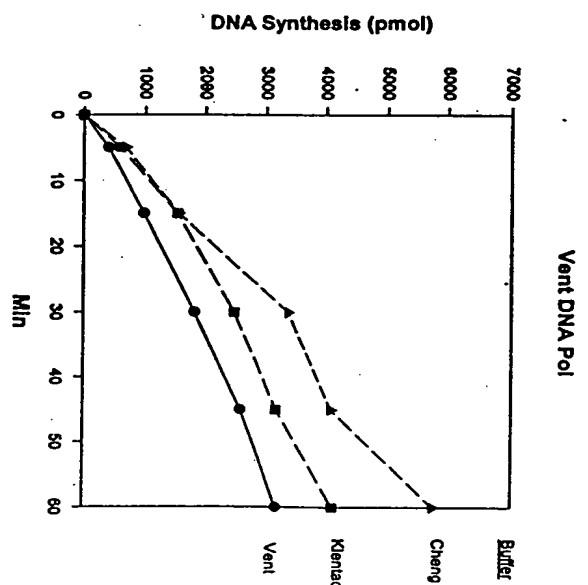
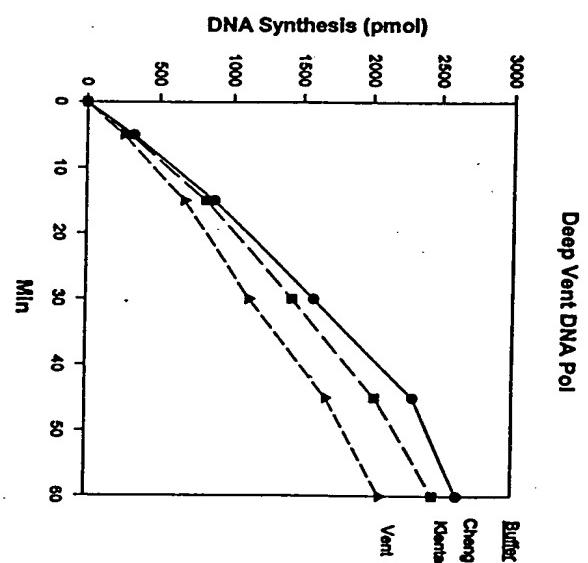
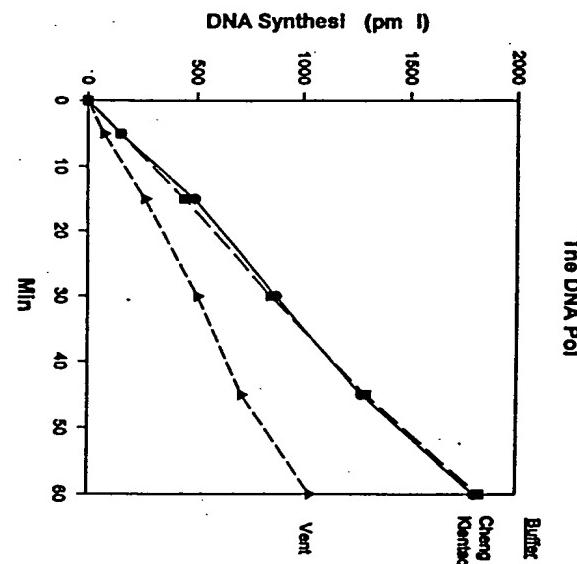
Record d by

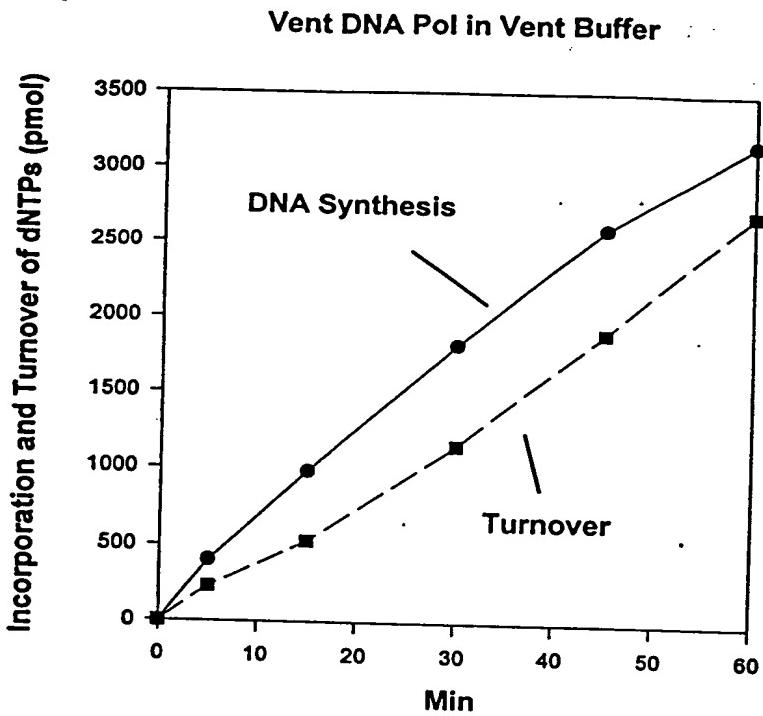
Date

115-94

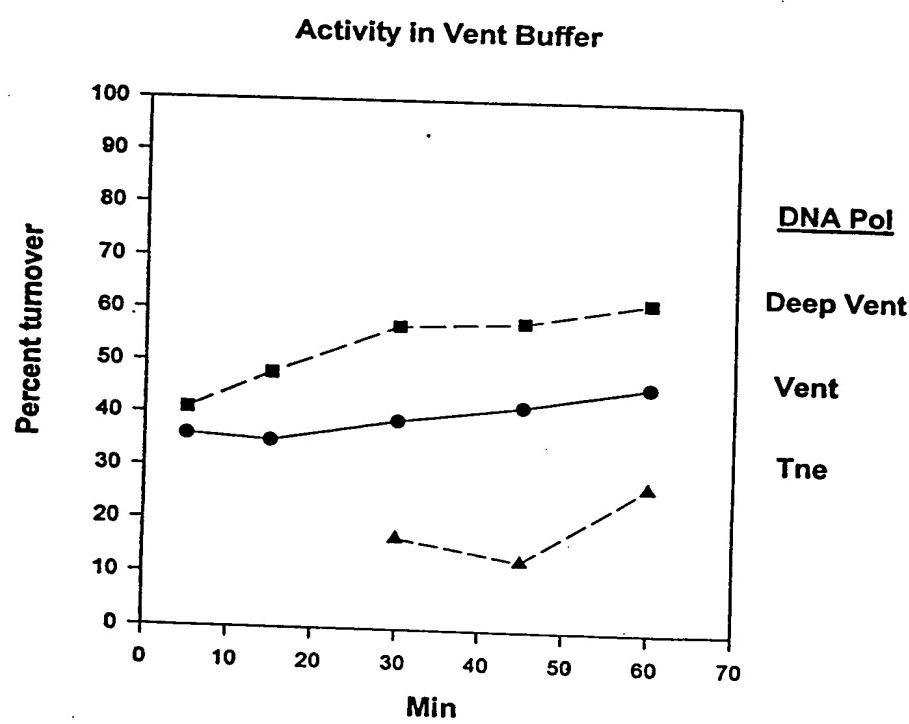
In such case, DNA synthesis is lower in 1
Prim degradation was highest in Vent

not turnover
by DNA synthesis
below



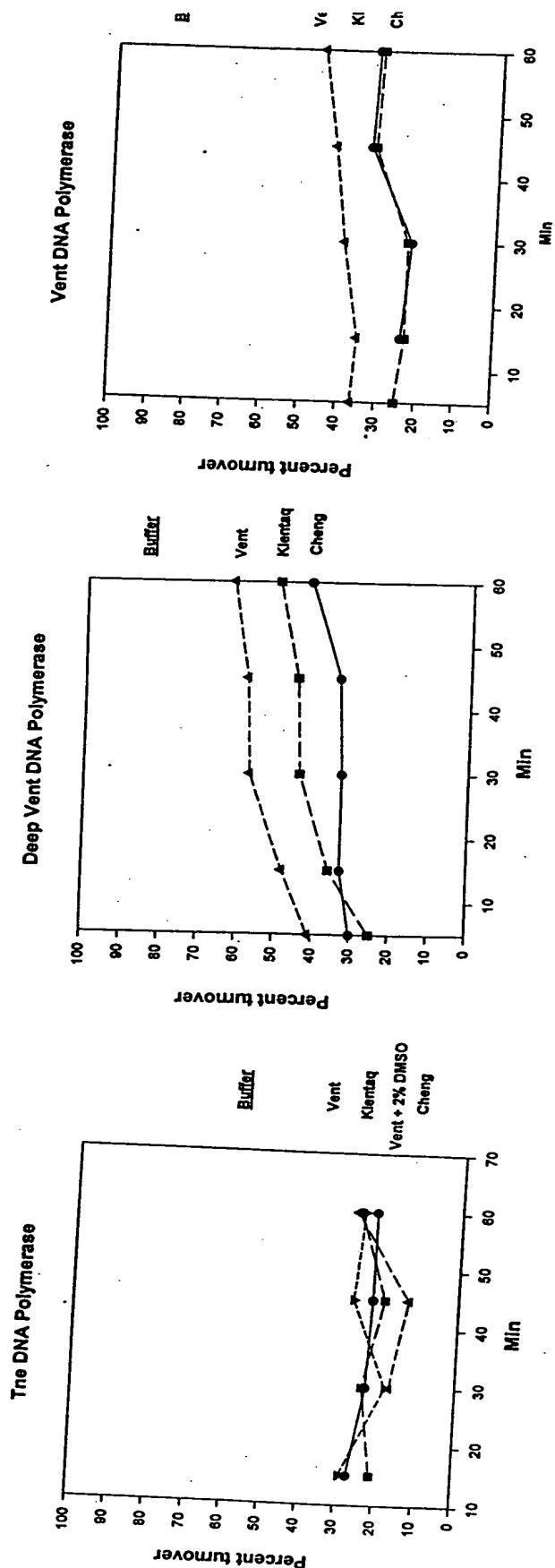


DNA synthesis
and turnover
to dNMP



$$\frac{\text{turnover}}{\text{incorporation} + \text{turnover}}$$

Deep Vent has higher turnover than Vent as expected. Tne is $\sim 2\times$ lower than Vent and Deep Vent



Effect of buffer on turnover is not large
compared to effect on primer degradation

Issued & Understood by me,
Cancer Research UK

Date
11/29/94

Initiated by
Recorded by

Date
11-5-94

32

Project No. _____
Book No. _____

TITLE New 3'-5' exo nuclease Mutant of T^d

From Page No. _____

4/13 -

Purpose: ~~See~~ Previous clone (P.129) of a 3'-5' exo nuclease mutant of Thermotoga neopolitana (Tn) proved not to produce over express a heat sensitive or no polymerase activity. Therefore Drs. ~~and~~ made a new clone. The purpose of this experiment is to screen pre + post heat kill for a polymerase activity. If activity is more thermal stable then proceed w/ a PET + (NH₄)₂SO₄ ppt.

3 grams of cells - suspend in 1ml of crack buffer - Sonicate with ~~minitip~~ micro tip ~~walkersan bath~~

Crack Buffer -

20 mM Tris pH 7.5

10 mM KCl

1 mM EDTA

5 mM BME

5% glycerol

A_{575} - .824 before crack

A_{575} - .198 after crack 6x 20 sec

minifuge setting 4

75% crack -

Save 400 μ l \rightarrow No heat treatment

Aliquot the rest of the cracked material to 2mL eppendorfs
heat Kill 10 min \rightarrow temp. @ 80° - 90° C -
note: temperature rose to > 90° in maybe up to 5 min

Spin in microfuge @ 14,000 x g 30 minutes -

Treat treat supernatant $< 90^\circ > 85^\circ$ - for 5 minutes -
spin in microfuge @ 14,000 x g 10 minutes -

To Page 1

Witnessed & Understood by me,

Date

Entered by

Date

May 20/95

6/20/95

Recorded by

6/13/95

Ag N - say for thermostable polymerase activity - 06/13/95

mix - TAQ premix - premade by A.G. -

add 11 µl / 500 µl premix
of 232 dCTP

$$\frac{10}{90} - \frac{1}{1000}$$

$\frac{1}{1000}$ [1]
 $\frac{2}{1000}$ heat treated

Dilution 5/4/95

$$\frac{10}{290} - \frac{1}{3000}$$

$\frac{1}{1000}$ [1] before heat treatment

$\frac{1}{1000}$ [2] mistake made - put 78 µl of premix should have only used
or 48 !!

incubate 10^1 @ 74°C in a heated water block - quench rxn w/
10 µl of 5M EDTA - spot 30 µl on 67°C

wash filters 1x TCA = 5'

3x 5% TCA 3'

2x Starch

dry + count in econofluor LSF

AM CPM1

1	Y1000 2994.00	H kill
2	2384.00	
3	Y1000 622.00	
4	888.00	
5	Y1000 3612.00	No H kill
6	5296.00	
7	Y1000 1234.00	
8	Y1000 1662.00	
9	964.00	
10	90094.00	
11	89736.00	
12	89120.00	
13	40.00	

S.A

27 Oct/95

First approx. - looks as though not
as much activity after
heat & kill - need to do
less dilutions to in order
to ascertain what exactly
is going on

Seeing host polymerase act. in No Heat Kill?

Repeat w/ $\frac{1}{500}$
 $\frac{1}{200}$ dilns.

MZ

01/20/95

T Page No. _____

is d & Understood by me,

May Tongo

Date

6/20/95

Invented by

Elizabeth Flynn

Date

6/13/95

Recorded by

20

Project No. _____
Book No. _____

TITLE _____

From Page No. _____

2/28/95 Tue

I set up digest DNA ppt.

① M13 mp 18, ② mp 19 and ③ T. nea/pSPORT

1. - 10. ca 3 added 100.0 μ l TE } to ppt
" " 10.00 μ l NaAc } DNA
" " 300.00 μ l EtOH }

2. Incubated on dry ice for ~5 min.

3. Cfg. for 10 min. @ room temp. (no ppt) &

4. no ppt., added 2.0 μ l Yeast/tRNA. (carrier molecule) Vortexed

5. incubated on dry ice for ~5 min. (supernate saved)

6. Cfg. for 10 min. @ room temp. (pellet was seen on mp 18 el)

7. added 200.0 μ l 70% EtOH to the pellet

8. Cfg. discarded supernate, air dried by putting tubes on the heat block.

II DIGEST set-up (to map Bam HI site)

- cut T. nea/pSPORT with Hind III, Bam HI, Xba, Nde I, Sst, EcoR separate

H_2O - 13.0 μ l enzymes - Hind III, Xba, Sst had REact 2 buffer

buffer - 2.0 μ l

T. nea/pSPORT DNA - 3.0 μ l

enzyme - 2.0 μ l

TV = 20.0 μ l.

- Bam HI, Nde I, EcoRI had REact buffer.

for Control: H_2O - 13.0 μ l
separate (REact 2) buffer - 2.0 μ l
enzymes DNA - 3.0 μ l

- Incubated @ 37°C

- ran on the gel on 3/1/95 (wkd)

Picture shown pg 21

To Pag N

Witness d & Underst od by me,

Date

4/1/95

Inv nt d by

Recorded by

Dat

4/12/95

ag N _____

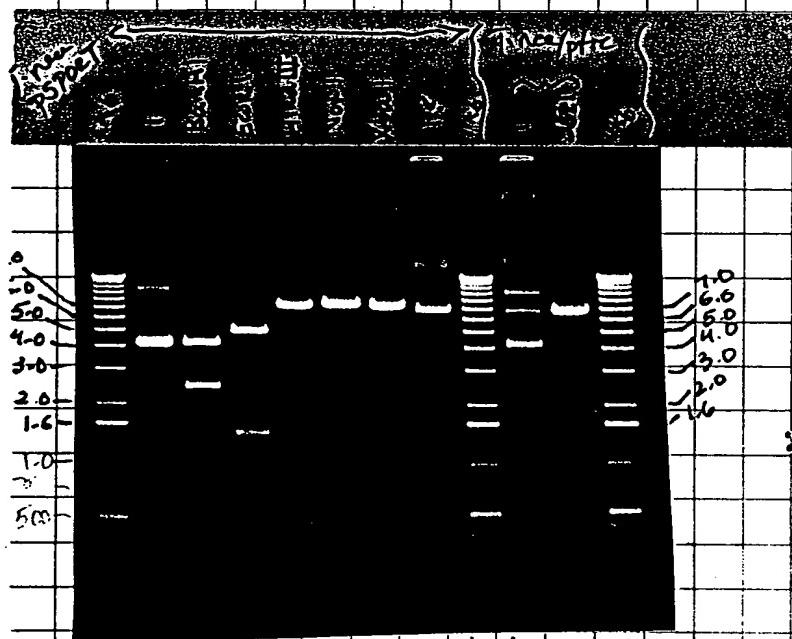
I DIGEST setup

- Cut pTrc/T.nea w/ Sst I enzyme

H_2O	- 11.0 μl	(Control: H_2O - 11.0 μl)
cT ₂ buffer	- 2.0 μl .	buffer - 2.0 μl .
T.nea DNA	- 2.0 μl .	DNA - 2.0 μl
Sst I	- 5.0 μl .	
TV	= 20.0 μl .	

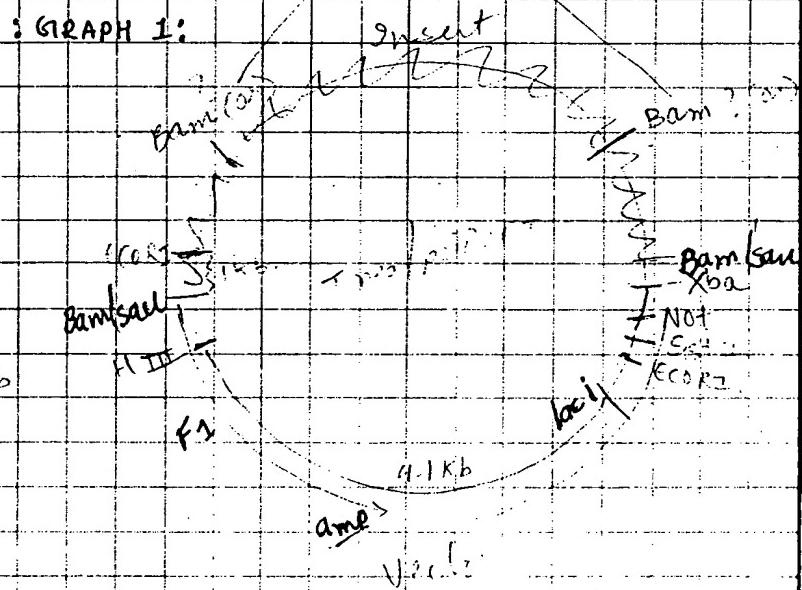
- incubate @ 37°C

- ran DNA on a gel on 3/1/95 (wed) picture shown below



Eco RI		Hind III	
3.210 kb (11.1 kb)	5.0 kb	1.0 kb	
2.5 kb (2)	1.5 kb		
1.0 kb	6.6 kb		
	5.8 kb		
	4.0 kb		
	3.0 kb		
	2.0 kb		
	1.4 kb		

: GRAPH 1:



: GRAPH 2: pg 23 of this book.

To Page No. _____

s d & Understood by me,

P. Polkay

Date

4/12/95

Invented by

Recorded by Dan

Date

4/12/95

84

Project No. _____
Book No. _____

TITLE

Turnover for Vent, Deep Vent -
(follow p. 61, 7)

From Page No. _____

H₂O
5 x Chey buffer

(A)

393 ~~455~~
133

(B)

784 464

(C)

489 476

10 x Klentag

10 x Vent buffer

Tag storage buffer

6.71

3.7 mg/ml activated

90

66.7

66.7

DNA

DATG-T-TP 10mM each

3.33

³²Pd ATP 10mCi/ml

1.021

Mg(OAc)₂ 50 mM

16 μl

MgSO₄ 100 mM

DMSO 100%

μl

0.655 ml

0.633

0.633.650 use 180

(1) (2) (3)

195 195 195

(4) (5) (6)

190 190 190

(7) (8) (9)

190 190 190

Tag storage buffer

4 4 4

4 4 -

4 4 -

Vent 0.02 μl

4

4

4

Deep Vent .02 μl

4

4

4

Taq 0.02 μl

4

4

4

prim 2.5°C, start by addition of pol 5' 6' 6' 6'

remove 15 μl to 5 μl 0.2 M EDTA → spot 1.5 μl on GFC and remove 5 μl to 5 μl Kill solution (20 μmol/ml DAPI 100 mM EDTA) at 9

0 5 15 30 45 60 min

spot 2 μl on DEI

resolve in 1m LiCl

* deletions of pol
name as P.J.

Results : see graph on P.J.

I fitness d & Understood by me,

Devena Balcar

Dat

11/29/94

Invented by

Record d by

Dat

11-9-94

T Page N

g N

(1)

14.4 ✓

✓

66.7 20

✓ (0.1 μl / 100 μl PCR ⇒ Cf = 0.05% Tissue 20/1000)

this makes up for no T_f₀ here - its present in Joe's long PCR Rxn.→ 27 ✓ (C_f = 50 pm early)→ 0.36 ✓ (220 × 10⁶ total cpm)✓ (1.2 mM Mg(OAc)₂)14 μl ✓ (1.2 mM MgSO₄ in Klenow buffer
(2 mM MgSO₄ in 1X Vent buffer))

(10)

19.4 ✓

(0.4 units total of each pol)

4

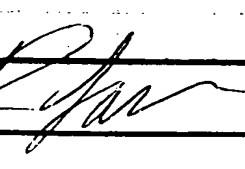
I & Understood by me,

Renee Polley

Dat

11/29/94

Invented by


Recorded by

Date

11/9/94

To Page No. _____

134

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

41

48 μl of pre mix aliquoted to pre labeled eppendorfs
unlabeled

1	4200	1	2
2			- post heat kill
3	4500	1	2
4			
5	4500	1	2
6			- pre heat kill
7	4500	1	2
8			

Incubate @ 74°C in a heated water block - for 10 minutes
quench w/ 10 μl of .5 M EDTA - spot 30 μl on GPC

Wash 1x 10% TCA 1% Pi 5'
 3x 5% TCA
 2x EtOH

dry + count w/ Econoflow...

USER: 2 ID: 32P

SAMPLE REPEAT:

H#: 1 AQC:N QCF

CHANNEL 1-LL:

DATA CALC: CPM.

HALF LIFE(DAYS):

SAM CPM1

1	8460.00	11.9
2	21680.00	19.8
3	8296.00	23.9
4	7486.00	28.3
5	16274.00	28.4
6	28614.00	30.1
7	8412.00	29.5
8	17912.00	29.7
9	2794.00	
10	S.A. 91294.00	

S.A. 57 cpm/pmol at

me

6/20/95

To Pag 1

Witnessed & Understood by me,

Date

Invented by

Dat

May Tong

6/20/95

R c rded by

Elizabeth

06/10/95

Page No. _____

12Y. SDS PAGE - 3'-5' examines the



EF off by gr

11/4 6/20/95

1	2	3	4	5	6	7	8	9	10
1 μg	3 μg	5 μg	10 μg	20 μg					1 M

[] []
one heat post heat

Kill Kill

uheat - @ 20 μg/ml - spun down sup loaded on gel
st heat @ .36 μg/ml

To Page No. _____

ssed & Understood by m ,

Date

Invented by

Date

May Long

6/20/95

Rec rded by

E. Flynn

06/14/95

Project No. _____
Book No. _____TITLE FY1 - 5.5gm crack

From Page No. _____

Purpose : to screen FY1 - 1 mutant Thr - one base point mutation phenylalanine to Tyrosine 1 for thermostable polymerase activity.

5.5grams cells - resuspend in 10mLg crack buffer - P.B2 ~1°

Divide in two 7.5mL Samples in 15mL Conical
Sonicate w/ microtip @ max output - 5
6x 20sec bursts

FY1	Betae - A ₃₉₅	.750	~ 57% crack
5.5g	After - A ₅₉₅	.320	

Sonicate again - 3 x 20sec bursts

before	A ₃₉₅	.750	~ 73% crack
After	A ₅₉₅	.200	

Divide Save 400mL - pre crack material.
Aliquat remainder of crack into 2mL eppendorf
incubate @ 87°C 11 minutes -
Spin in microfuge 20 minutes @ 14,000 \times g

Decant and save Supernatant \Rightarrow assay for
thermostable Polymerase activity -

P.B2

To 500 μ l g TKA Pre mix add 1.1 μ l g d₁ CTP

48 μ l of premix / rxn - 1n2 μ l of
diluted samples added incubate @
74°C in a heated water bath block -

for 10'. Quench rxn w/ 10 μ l of
.5M EDTA - spot 30 μ l g GF/C
Wash 1X w/ 10% TCA 1% P.i @ 5'
3X w/ 5% TIA @ 3'
1X w/ 3% TGA

dry + count -

To Page N

Witnessed & Understood by me,

Date

Inventor by

Date

E. Flynn

05/16/95

May Tongo

6/20/2023

Recorded by

Page N. _____

USER: 2 ID: 32P
 SAMPLE REPEAT:
 H#: 1 ADC:N QCP
 CHANNEL 1-LL:
 DATA CALC: CPM.
 HALF LIFE(DAYS)

SAM CPM1

1	5722.00
2	7676.00
3	2608.00
4	4686.00
5	10454.00
6	19114.00
7	5850.00
8	11594.00
9	2976.00
10	90102.00
11	88418.00

Post
Heat Kill5.8
5.0

5.4 u/l

loss of 70%?

Pre Heat Kill

15.9 u/l
17.1 u/l

17.0 u/l

1/20/95 Appears to have (very high background) +
 lost activity after heat kill?
 & maybe mostly post pol activity in pre heat kill?

Bradford

Slope - .0555 O.D./ug

in O.D.

u/l

Heat Kill T41 - 40 .474 * too ↑ .2 ug/l

20 .333

.289 ug/l

Pre Heat Kill T41 - .259

.439

23.3 ug/l

Post Heat Kill

.303

.136 ug/l

3'5'ero mut

19.8

Run 12x SDS PAGE - see D 38

1 2 3 4

v

To Page N. _____

Is d & Understood by me,

Date

Invented by

Date

May Longo

6/20/95

Recorded by

6/16/95

Project No. _____
Book No. _____

138

TITLE PY1 + 3'-5' exo mutant SDS PAGE

From Page No. _____

06/1

12% SDS PAGE.

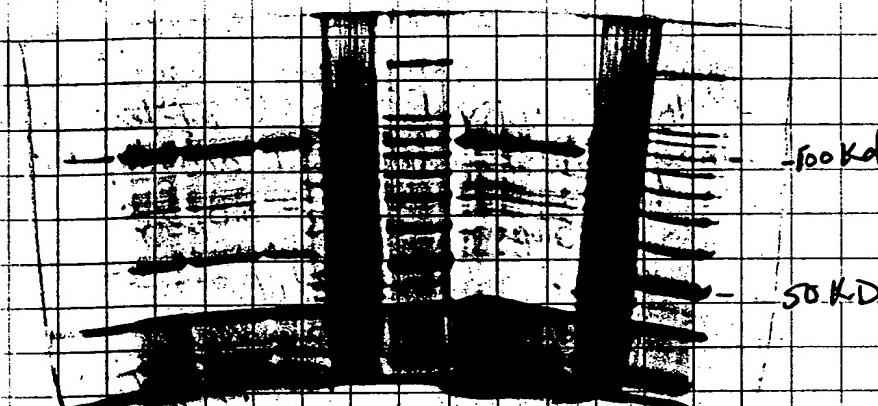
1 2 3 4 5 6 7 8 9 10

1 6μg 4μg 2μg 60μg M 4μg 2μg 60μg M

FY1
post heat
kill

FY1
preheat
kill

3'-5' exo M. preheat
postheat kill 3'-5' exo -
kill



DRF 6/20/55

GFF
C

preheat kill - spun down sup loaded on gel

To Page #

Witnessed & Understood by me,

Date

Invented by

S. Flynn

Date

May Longo

6/20/95

Recorded by

06/16/95

22

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

3/2/95

- ① 1 kb ladder ② T-neal/pSPORT uncut, ③ Sst, ④ Sst/sph, ⑤ Sph, ⑥ 1 kb ladder. (from 263 added loading dye, electrophoreses @ 190 V

- digested double digested BamHI/SphI (to map the Bam site T-neal

H₂O - 14.0 μl

control i - H₂O - 14.0 μl

(REact6) buffer - 2.0 μl

(uncut) buffer - 2.0 μl

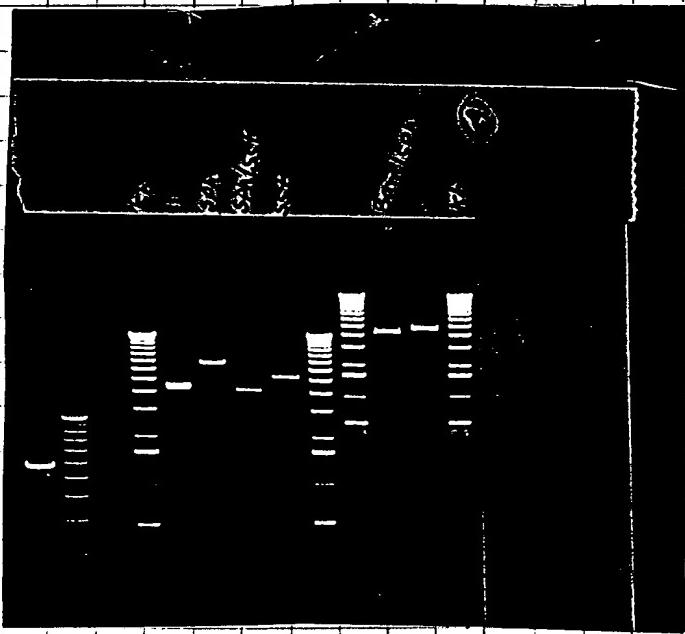
'yodib' (T-neal/pSph) DNA - 2.0 μl

DNA - 2.0 μl

(Bam/sph) enzyme - 1.0 μl ea.

TV = 20.0 μl.

Incubated @ 37°C for 300 min. (15 min.)



To Pag 1

Witness d & Understood by me,

Bob Staub

Date

4/12/95

Invented by

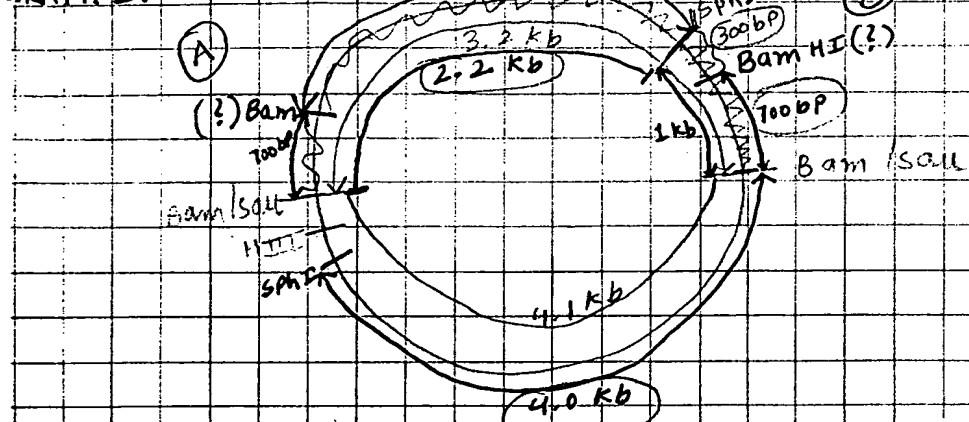
R c rd d by *[Signature]*

Date

4/12/95

age No. (from pg. 2)

GRAPH 2:



Bam Sph

(A)

4.0 kb

700 bp

1 kb

1.5 kb

3.2 kb

1.1 kb

1.5 kb

Bam Sph I

(B)

4.0 kb

2.2 kb

300 bp

700 bp

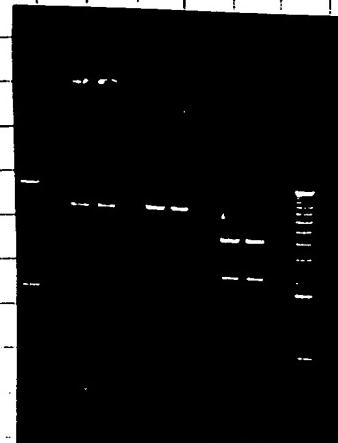
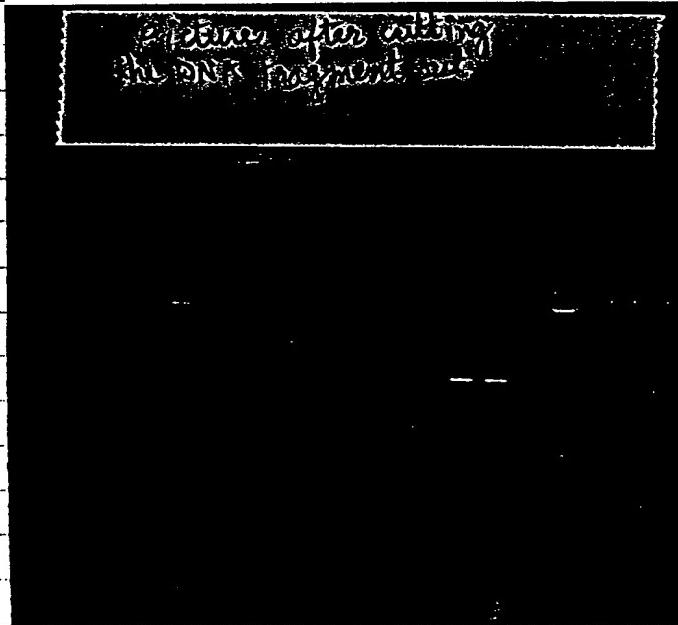
3/2/95

Cloning mp 18 w/ T.nea/pSPORT E1 mp 19 w/
T.nea/pSPORT

~~3/2/95~~

Thurs.

Picture before
cutting the
DNA fragment.



DNA GENE CLEAN!

To Page N _____

ss d & Understood by me,

Solcyn

Date

4/14/95

Invent d by

Recorded by

Date

4/12/95

122 12/6/94

Project No. _____
Book No. _____

TITLE PMO 9 / Tag / Tag + DV / F+

From Pag. No. _____

Purpose: - To check band primers with 2 v of engg.
+ 3 step cycle

- Repeating expk. page 116 - 118 but used

non dv forward & reverse
47.3 pm 52.9

12 x 9 each with Tag or Tag + D.V

10 x bigger - 494.2
dNTP 60

Temp
primer 2 2.4
11.5
1 12.7
enggme 4.8

499.4

60.0

12.0

2.4

11.5

12.7

12.0

200 μM

200 pg

1 μM

KIT bsp

enggme

TDV. 1.0 ±

4.5 μl of cock tail + 5 μl of Mg different conc.
1 - 3 mgy

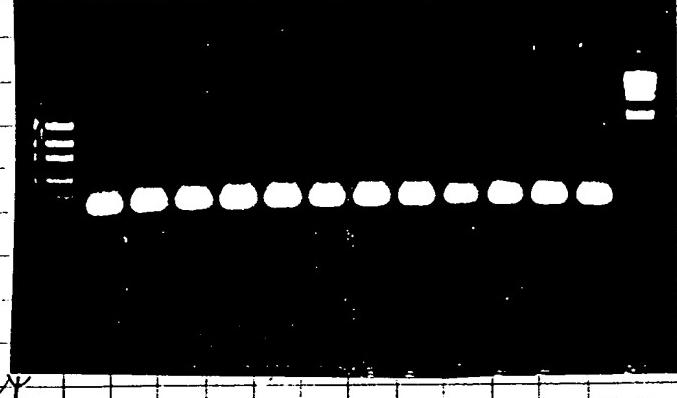
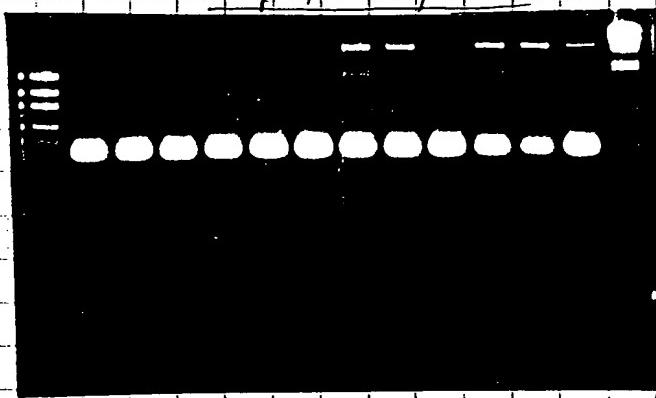
Cycles 94°, 3'

30(92°, 30", 56", 30", 72", 3')

72°, 10" → 4° heat

Tag + supplement

Tag alone samples done



0 1 1.5 2 2.5 3 mgy 3 mgy A

To Page 1

Witnessed & Understood by me,

Date

Invented by

Date

12/6/94

Recorded by

12/6/94

The Librarian

age No. _____

Results: Tag alone / 3 step cycle / ~~Exe~~ no - dv didn't work again.

Tag + D.V : as usual even with 1U 2 mM - 3 mM works.

For some reason these new primers at 1 pmr conc / 50 pmL gives best 9 primer drivers.

Judy's 2 step cycle works with Tag 2 U.

Not transformed anything yet.

To Pag. No. _____

Signed & Understood by me,

Date

12/19/94

Invent'd by

Date

12/19/94

Recorded by

Dr. Subramanian.

Fr	m	P	Book No.	Turner	Title	Inv	nted by	Winn
0	1		543.00	BK60	(14)	61	269.00	
15	2	V	650.00	110	24	62	7412.00	679
30	3		1014.00	486	22	63	16953.00	9553
45	4		1485.00	971	34	64	36825.00	3374
60	5		2627.00	2148	32	65	44610.00	4087
7	6		3187.00	2725		66	62771.00	5752
8	7		525.00	BK60		67	241.00	
9	8	DV	662.00	141	30	68	3518.00	322
10	9		948.00	436	33	69	9506.00	871
11	10		1271.00	769	33	70	17320.00	1587
12	11		1677.00	1188	34	71	25050.00	2296
13	12		2340.00	1871	42	72	28643.00	2625
14	13	TW	624.00	BK60	(32)	73	324.00	
15	14		694.00	72		74	1974.00	151
16	15		796.00	177	27	75	5340.00	489
17	16		880.00	264	23	76	9478.00	869
18	17		976.00	363	22	77	13880.00	1272
19	18		1110.00	501	22	78	19753.00	1810
20	19		805.00	BK60	775 AVE	79	321.00	
21	20	V	977.00	192	25	80	8826.00	5PP
22	21		1409.00	467	23	81	23029.00	1533
23	22		1803.00	762	23	82	37324.00	2485
24	23		2832.00	1335	32	83	47661.00	3175
25	24		6299.00	1883	31	84	61758.00	4112
26	25		774.00	BK60		85	404.00	
27	26		918.00	99	25	86	4493.00	299
28	27	DV	1406.00	415	36	87	12238.00	815
29	28		2277.00	1118	44	88	21497.00	1431
30	29		2989.00	1651	45	89	30491.00	2030
31	30		4085.00	2472	50	90	36800.00	2450
32	31		777.00	BK60	(12)	91	214.00	
33	32		813.00	21		92	2257.00	150
34	33	TW	947.00	121	21	93	6671.00	444
35	34		1136.00	263	24	94	12685.00	845
36	35		1204.00	314	19	95	19429.00	1294
37	36		1631.00	633	26	96	27534.00	1855
38	37		919.00	BK60	922 AVE	97	239.00	
39	38	V	1284.00	231	36	98	7128.00	404
40	39		1754.00	530	35	99	17335.00	871
41	40		2728.00	1150	39	100	32171.00	1821
42	41		3910.00	1903	42	101	45795.00	2592
43	42		5168.00	2704	46	102	56065.00	3174
44	43		924.00	BK60		103	318.00	
45	44	DV	1205.00	180	41	104	4474.00	253
46	45		1892.00	617	47	105	11839.00	670
47	46		3234.00	1472	57	106	19756.00	1119
48	47		4572.00	2325	58	107	29674.00	1170
49	48		6365.00	3467	62	108	36540.00	2069
50	49		863.00	BK60		109	261.00	
51	50	TW	901.00	—		110	1566.00	74
52	51		953.00	20	(7)	111	4647.00	263
53	52		1083.00	103	17	112	8879.00	503
54	53		1085.00	103	13	113	12496.00	707
55	54		1529.00	386	27	114	18327.00	1037
56	55		984.00	BK60		115	295.00	
57	56		891.00	—		116	1709.00	80
58	57	TW	1067.00	92	29	117	4261.00	214
59	58		1086.00	104	18	118	8343.00	49 Page 1
60	59		1336.00	264	67	119	12504.00	708
			1467.00	347	25	120	18443.00	1048

Witt

Dorcas Polkup

" 129194

Recorded by

1/9-94

Calculations

Project N _____
Book N _____

87

g N

JAMP BKGD

1. Chey mix = 564 ave
2. Klenet mix = 785
3. Vent mix = 922

spot

Chey

Klenet

Vent

$$75821 \text{ cpm} \left(\frac{5 \text{ mol Rxn vol}}{2 \text{ L spotted}} \right) \left(\frac{200}{185} \right) \left(\frac{1}{2500 \text{ pm}} \right) \left(\frac{1}{4} \right) = 194 \text{ CPM at pm}$$

$$26.7 \text{ cpm/pmol}$$

$$314 \text{ CPM/pm}$$

$$\text{pmol incorp} = \frac{\text{CPM}}{(200 \mu\text{L Rxn})}$$

$$\frac{\text{CPM}}{\text{CPM/pmol}} \frac{(200)(20)}{(15)(15)}$$

$$\text{pmol turnover} = \frac{\text{CPM - BKGD}}{200 \mu\text{L Rxn}}$$

$$\frac{\text{CPM - BKGD}}{\text{CPM/pmol}} \frac{(200)(10)}{5(2)}$$

$$\% \text{ turnover} = \frac{\text{pmol turnover}}{\text{pmol turnover} + \text{pmol incorp}}$$

121 75821.00
122 104512.00

To Page No. _____

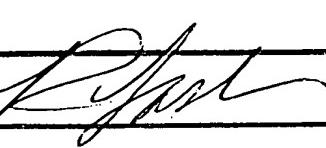
seen & Understood by me,

Suzanne Polley

Date

11/29/94

Invented by



Date

11-10-94

$$\text{DGI} + \text{Ag}_2(\text{NH}_4)_2\text{S} \text{ ppt.}$$

Project No. _____
Book No. _____

in to continue with purification - following the
same protocol as for wild type Trc - p.108. J

$$11 - 6.8 \text{ mL} \quad (.05)(6.8) = 2M x \\ 3.8 \quad x = 174 \mu\text{L g 201 KCl}$$

$$\text{exd} = 4.8 \text{ mL } 3.8 \quad (0.05)(4.8 + x) = 2Mx \quad x = 97.4 \text{ mL of } 2M \text{ KCl}$$

$$(.40\%) (6.8 + x) = 10\% \cdot x$$

$$291 \mu\text{L} = x \quad x = 291 \mu\text{L} \cdot 10\% \cdot DEI$$

$$(1.40)(3.9 + x) = 10\gamma \cdot x$$

$$14.3 \mu\text{L} = x$$

$$x = 14.3 \mu\text{L} \text{ g } 10\gamma \cdot \text{DET}$$

Make each of Anal. 50 mM KCl. Slowly add 20H or 10'.
PEI sol'n to a Anal [3 of .4']. vortex - let shake
30 minutes @ 4°C. spin in 2mL eppendorfs in micro-
centrifuge 20 minutes @ 4°C - Save Supernatant.

(eO): $(\text{Nb}_y)_2\text{Si}_y$ fractionation

$$TY1 \quad \frac{3\text{g solid}}{100\text{ mL}} = \frac{x}{4.8\text{ mL}} \quad 2.45\text{ g}$$

$$3.5 \text{ g} \times 0. \frac{36 \text{ g}}{100 \text{ mL}} = \frac{x}{3.5 \text{ mL}} \quad 1.26 \text{ g}$$

Vortex - let shake 30 min @ 4°C
Spin in SS-34 - 70,000 x g - -
Decant + Save Supernatant - pellet s

To Page No. _____

sed & Understood by me.

Date _____

Invented by

Date

Many songs

6/20/55

Recorded by

06/10/95

Project No. _____
Book No. _____TITLE 2mL TDSO Heparin

From Page No. _____

Bump Heparin with .5M NaOH - wash w/ 1Ld extension
 Equilibrate w/ Buffer A - Buffer B - Heparin

25mM Tris pH 7.4
 10% glycerol
 5mM BME
 1mM PMSF
 .1mM EDTA
 10mM KCl

conductivity - 1.2 mS

A.S.

25mM Tris pH 7.4
 10% glycerol
 5mM BME
 1mM PMSF
 .1mM EDTA
 1.5M KCl

TY-1 - Dissolve "Pellet" in 10mL of Buffer A

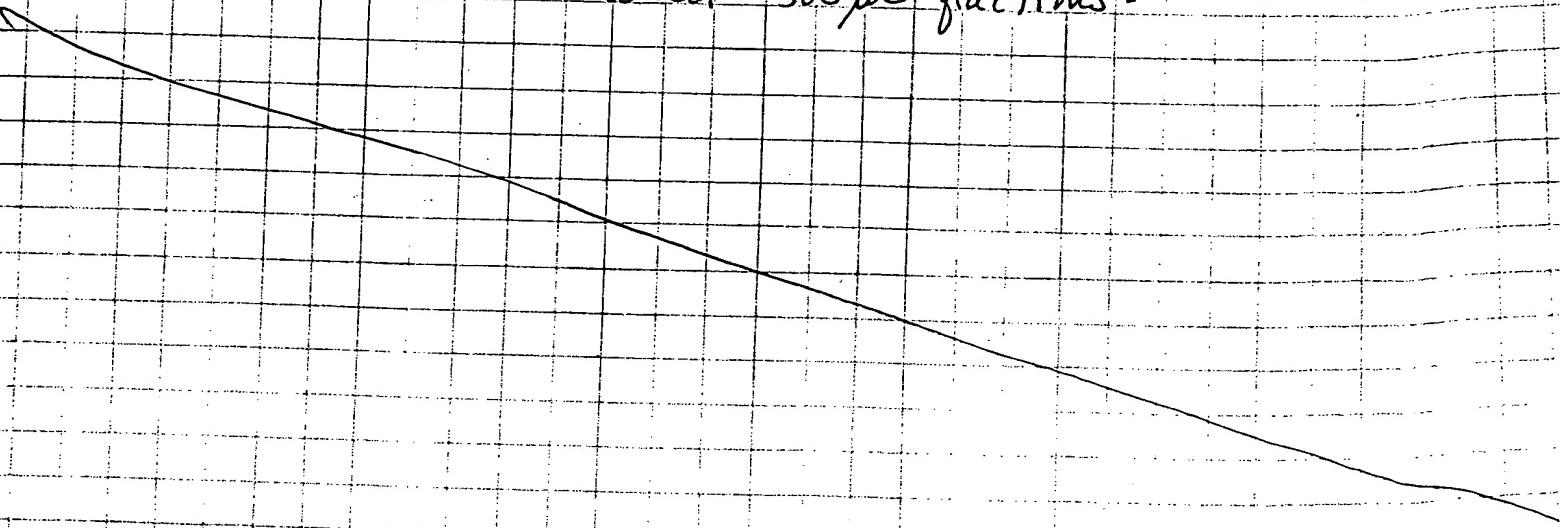
4.5mS - conduct

Add 30 mL additional of Buffer A

2.1mS - conduct

Load 9 35mL on 2mL TDSO Heparin @ .75mL/min
 collect flow through material - wash to base line -

Gradient Program - 0 - 100% B @ .5mL/min - 20mL linear
 wash 100% B - 10mL - @ .5mL/min
 collect 500μL fractions -



Witnessed & Understood by me,

Dat

Inv nted by

To Pag

Munir Farooq

6/20/25

R corded by

Date

6/15/25

ag N. _____

Mix Rxn

6/15/95

lock

SMTAPS

50 mM MgCl₂

2M KCl

1M DTT

10m M dNTP's

ct. Salmon testes

For 20mL

1mL

800 μ L500 μ L200 μ L400 μ L

5 mL

12.1

- 1.1 mL DCTP
Vial

20mLs

Migrant 500 μ L / tube store in -20°C freezer - yellow tubes -

To Page No. _____

ssed & Understood by me,

Date

Invented by

Date

Nancy Longo

6/20/95

Recorded by

6/10/95

Project No. _____
Book No. _____TITLE Heparin - TX-1

06/15

From Page No. _____

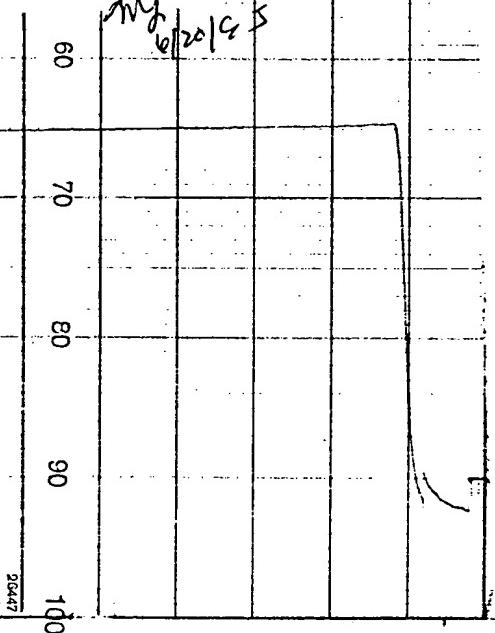
SAM CPMI

S/X

TX-1

1	115552.0052
2	53328.0054
3	9146.0056
4	4556.0058
5	1260.0059
6	3744.0060
7	1028.0061
8	574.0062
9	536.0063
10	346.0064
11	730.0065
12	438.0066
13	348.0067
14	21268.00Load
15	668.0044
16	372.0046
17	866.0048
18	74836.0050
19	146.00

Pool 47-55 dialyzed in QUSO Buffer A

MP
6/20/95

Pharmacia LKB Biotechnology

24 Jul Rxn
1ul pack
Sample -
incubate @
fn 8° min
w/ 10 μl of S
EDTA - SP
20 μl on t
wash
5' 1x 10' TCI
3' 3x 5' T.
2+ S to
dry + cool
ecoflower

Pool - 49
dialyze 0,
in again
QUSO Buff
See p. 144

6/20/95

To Page N

Witnessed & Understood by me,

Date

Invented by S. Flynn
Recorded by

May Tong

6/20/95

Date

06/16/95

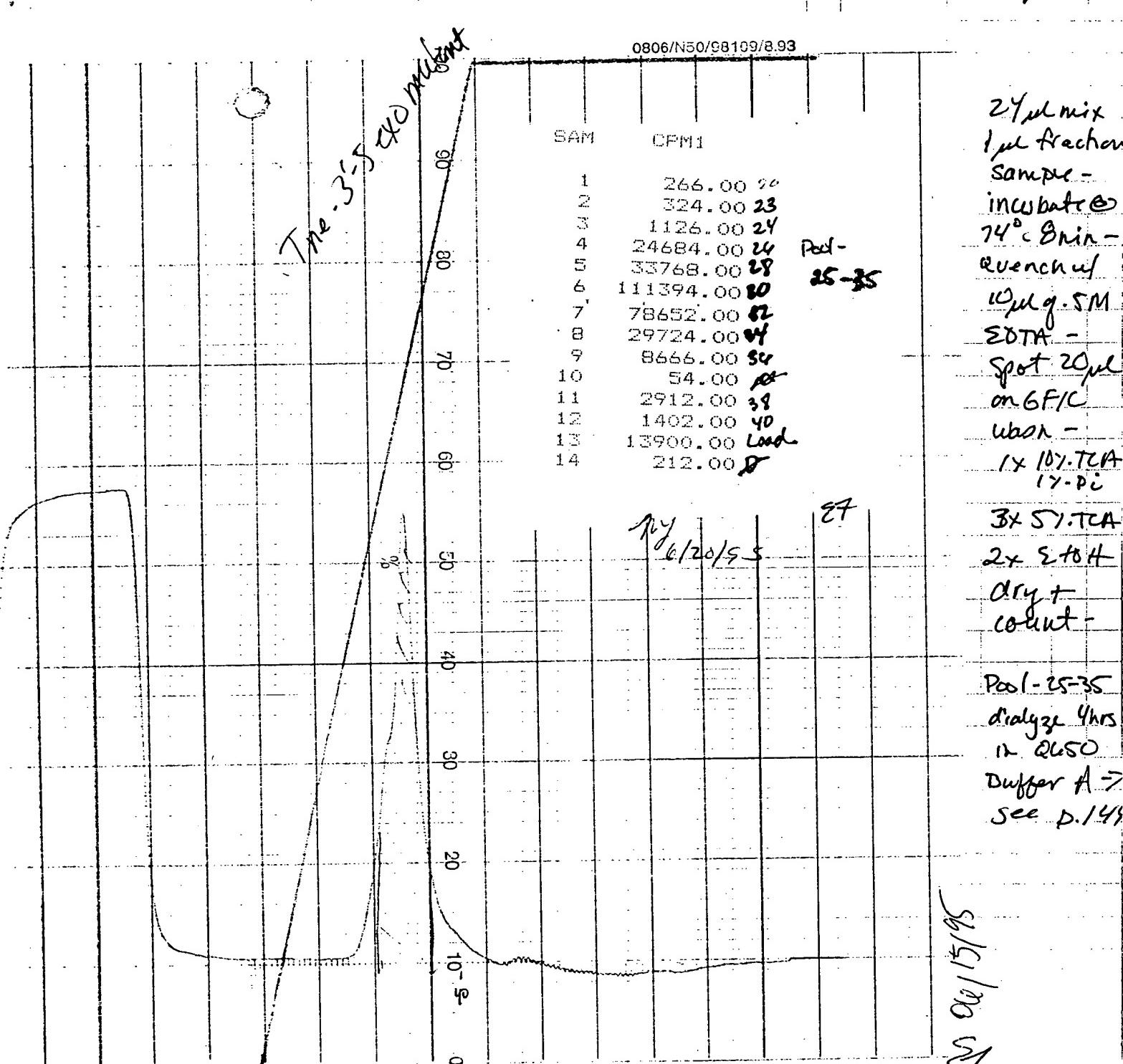
Hepairin 3-5exo mutant

Project No. _____
Book No. _____

143

ag No. _____

06/15



technology

Code No. 18-1001-44

To Page No. _____

signed & Understood by me,

May 10/95

Date

6/20/95

Invented by

Elgaust Ky

Date

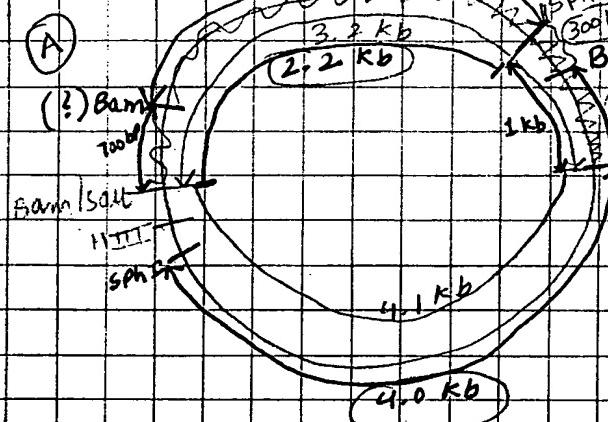
6/10/95

Project No. _____

Book N. _____

Ag No. (front pg 2)

RAPM 2:



(Cloning mp 18 w/ T-neo/pSPORT E1 mp 19 w/
T-neo/pSPORT

Bam I scall

(A)

4.0 kb

700 bp

1 kb

1.5 kb

3.2 kb

1.7 kb

1.5 kb

Bam I Sph I

(B) 4.0 kb

2.2 kb

300 bp

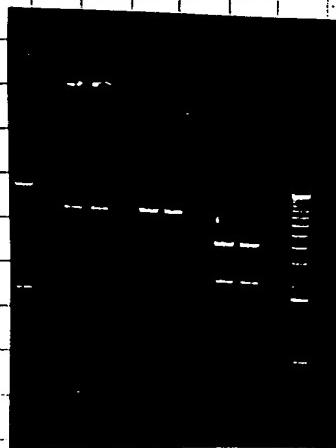
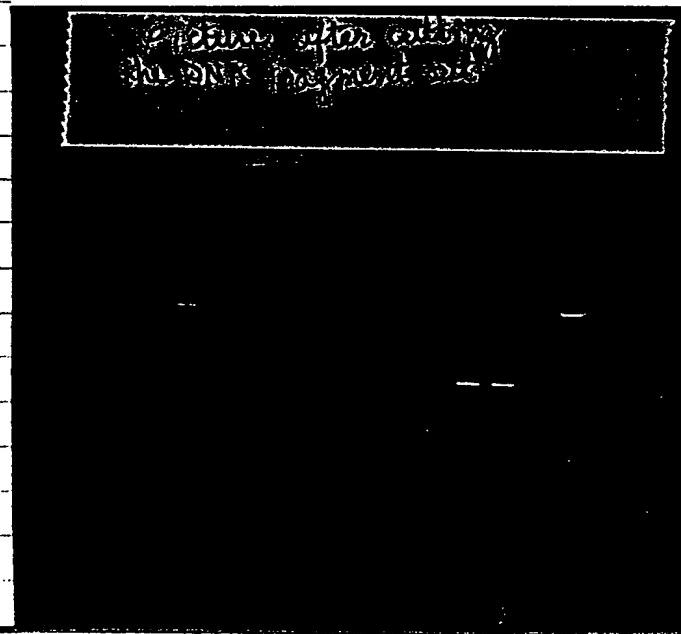
700 bp

3/2/95

3/2/95

Thurs.

Picture before
cutting the
~~RNA~~ DNA
fragment.



DID GENE CLEAN.

To Page No. _____

s d & Und rstood by me,

Powers

Date

4/14/95

Invented by

Recorded by

Date

4/12/95

Project No. _____
24 Book No. _____

TITLE _____

From Page No. _____

GENE CLEAN

- (cut)
- Mixed mp18 with *T.nea* pSPORT cut w/ Sst IspH 3/2 tube
 - " mp19 with " " " " 3/2 tube
 - added 700.0 μ l NaI to each 2 tubes. Vortexed
 - put the tubes in 55°C heat block to melt agarose
 - after agarose melted, added 5.0 μ l glass milk to both tubes
 - incubated both tubes on ice for 5.0 min.
 - cent. both tubes (quick spin)
 - discarded supernate & washed pellet 3x with New Wash b
 - added 14.0 μ l dH₂O to each tube
 - quick spun, discarded pellet & saved supernate

Set-up Ligation

→ (mp18)(mp19)
DNA - 14.0 μ l
(ligase) 5a buffer - 4.0 μ l
ligation - 2.0 μ l.
TV - 20.0 μ l.

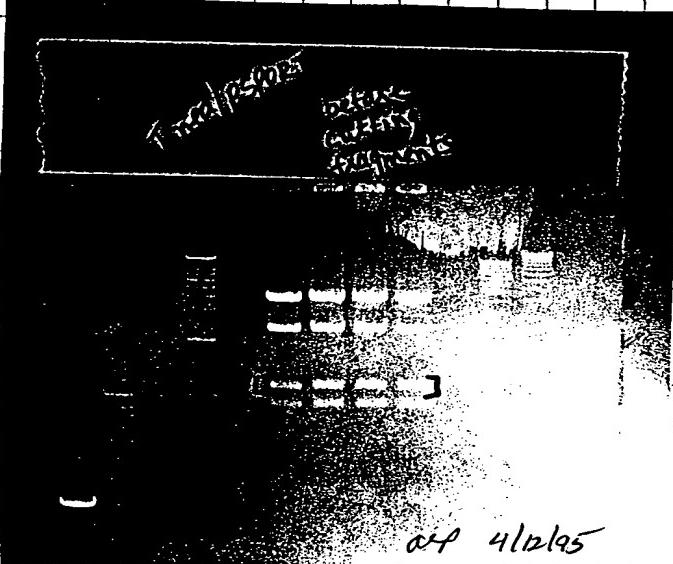
- incubated both

Infection Cells

(1) 100.0 μ l Competen
→ 3.0 μ l DNA (from ligation)

amp 4/12/95

3/12/95



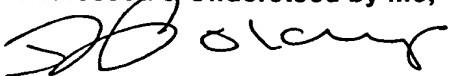
(2) incubated on ice for 30 min.
(3) heat shocked @ 42°C H₂O bath for 35 sec.

- melted 0.7% 2x YT top agar, added 4.0 ml to 6 different glass tubes & put the tubes @ 55°C heat block

didn't work

T Pag N

Witnessed & Understood by me,

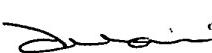


Dat

4/12/95

Inv nt d by

R c rded by



Dat

4/12/95

Project No. _____

Book No. _____

TITLE _____

124 12/6/94

From Page No. _____

Purpose: To try the new scheme! in 8 days! 10 hr.
18 hands!!!

C Vector prep: puc 19 - 100 µg *
0.45 µg/ λ packed from several tubes

- restricted with Dal II (1) - 1 hr = 3 hr
Eco RI & Barn HI (2) 1 hr = 3
Dal II (3) 2 hr = 2

succesively at 37°

LTI puc 19 100 µg 225 µl

NIEB buffer 4. (10x) 40

NIEB Dal II (24U/ λ) 10

TE 125

5 µg point
1.25 µg needed

LTI AKT 403 (Eco RI)

20

74121 Barn HI J 20

Co 6/1

440

4.5 µg 1.075 µg

LTI Df(3 \times 7403)

30

BMG 101 J

6U/1x

5' A'C puc 5' T-3' \ 5U/1x - 470 2 hr at 37°.

3' T G puc A 5' / 5U/1x

Run a b.c on minigel along with control puc and

candida 1/1ml in marker.

5 µl of a, b & c, mixed puc 0.5 μ = 0.225 µg

Point (C) stored at 4°c overnight.

To Page N

Witnessed & Und rstood by me,

Date

Invent d by

Date

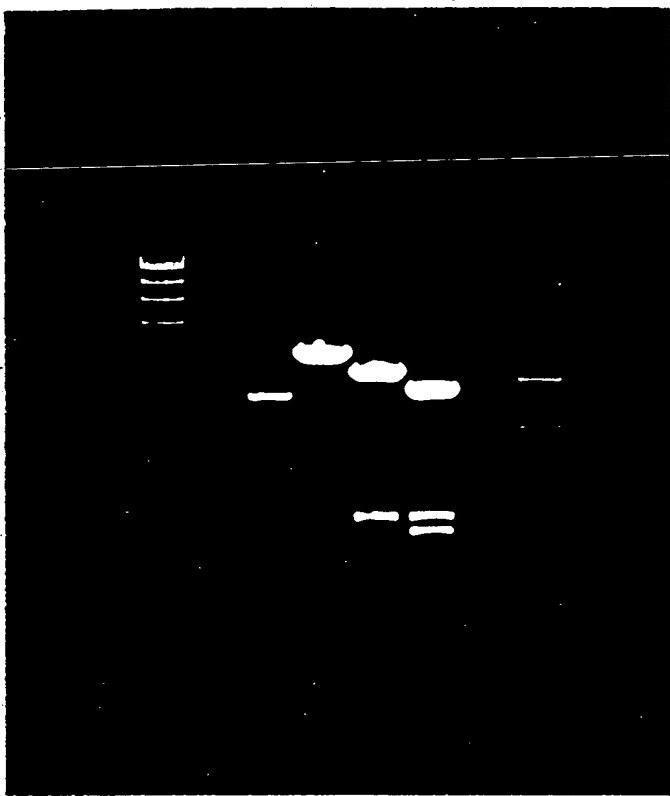
12/6/94

Recorded by

K. Libermann

12/6/94

age No. _____



unbuf Aat ECO RI
puc I Bam HI Apl 3.

puc = 2686 bp

DNA II @ 2617 + Apl II @ 806 = Vector = 1811 bp. 0.6742 / 1
rest = 875 bp 0.325 / 1

DEAE paper containing the vector band, washed in S/M wash
first 10 mM Tris 8.
5 mM EDTA

ethanol precip (Taq) 1m = strong /+

To Pag No. _____

ssed & Und st od by me,

Date

Invent d by

Date

R. S. S.

12/12/94

R c rd d by

R. S. S.

12/12/94

126 12/7/94

Project No. _____
Book No. _____

TITLE

puc / F&R non dv transform

From Page N

- Purples To transform puc / F&R non dv plasmids.
- amplified, ethanol psp'd, add with Dalt II again & hybridized with Dalt II
 - 2⁵ μl of ligation mix transformed 100 μl DH5α max E. coli.
 - plated 25, 50, 100 μl from each reaction (from 5 Test tubes + different Rx's: all prepared by Judy are

(1)	Tag, 1.5 mM Mg	25	17	-	
	(395)	50	9		all blue
		100	17	-	no white
(2)	Tag, 2 mM Mg	25	2	-	
	(396)	50	1	-	
		100	3	44	?
(3)	Tag + D.V., 1.5 mM Mg	25	10	6	60 20] 6
	(403)	50	25	17	68 20] 6
		100	24	13	54 20] 6
(4)	Tag + D.V., 2 mM Mg	25	136	40	29] 34
	(404)	50	223	82	37] 34
		100	405	141	35] 34

Result: Tag + D.V. unusually high error - mutation

Tag alone # of colonies too low, but whatever is there not much selective pressure.

Nothing makes sense.

To Page N

Witnessed & Understood by me,

Date

Invented by

Date

12/8/94

Recorded by

R. Subramanian

12/8/94

AT Gary carried Project No. _____
90 ml at Frederick Book No. _____

TITLE Lot # EKBT1 Repeat first assay QC for v12d
done on P 61 (yes)

From Page No. _____

amplifast lot # 9957 for control

lot EKBT1 is ~ 401 u/ml based on P.61

1. starting dilutions of EKBT1^o

1:80 (estimate Cf = 5 u/l)

lot EKBT1 5 u/l

Total storage buffer 385 u/l actual is 4.03 u/l

$$V_f = 400 \mu\text{l}$$

1:160 (estimate Cf = 2.5)

5 u/l

795 u/l actual is 2.01 u/l

$$V_f = 800 \mu\text{l}$$

2. 1/600 dilutions

serial dilution # 1-6 7-12 13-18 19-24 25-30 31-36 37-42 43-48 49-54

I II III IV V VI A-1 A-2 A-3

1:80 dil

3 3 3

3 3 3

3 3 3

1:160 dil

3 3 3

3 3 3

Amplifast 5 u/l

Vortex 5 s

lot # 1

use from 20 and 40 ml

dilution buffer 1797 u/l

$$V_f = 2000 \mu\text{l}$$

$$V_f = 1800 \mu\text{l}$$

dilute I - A-3 as shown for I below:

3. Serial dilutions

dilution buffer

dilute I - III and assay
then dilute IV - VI and assay
then dilute A-1 - A-3 and assay

serial dilution #	1	100 λ	7300 λ
2	100 λ	7300 λ	
3	100 λ	7300 λ	
4	100 λ	7300 λ	
5	100 λ	7300 λ	
6	1m1 of I	7300 λ	

SA I-III = 45 μl assay mix + 5 μl dil buffer, do same for IV-VI

spot 4x 5 μl on GFC in one aquasol

Blank is 45 μl assay mix + 5 μl dil buffer \rightarrow spot on GFC along with other To Pag

Witnessed & Understood by me,

Date

1/6/95

Invented by

Date

11-15-94

Recorded by

age No. _____

55-57 = Blanks for I-II, IV-VI and A1-A3 respectively

58-61 = SA for I-III

62-65 = SA for IV-VI

Results:

using amphetaq lot #9957 here gives a unit value of ~~320 u/l~~ 323.4 u/l compared to 401 u/l (found on P61, 10-1-94)

s d & Und rstood by m ,

enriched Polycap

Date

1/6/95

Invented by

Recorded by

Date

10-15-94

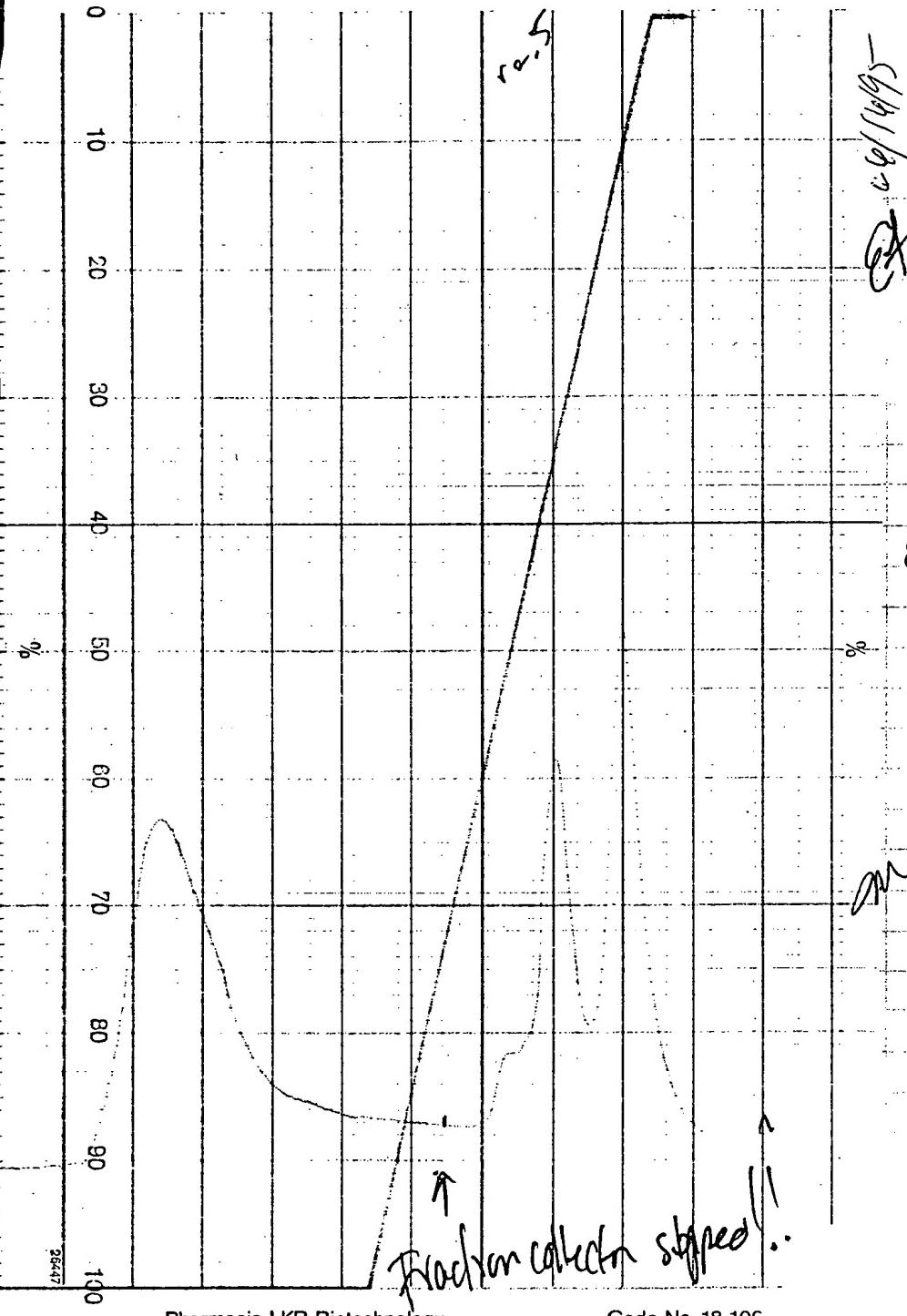
To Page No. _____

146

Project No. _____
Book No. _____

TITLE FY-1 Q GSD M - 2 ml

From Page No. 146



Q Buffer A -

25mM Phos - pH 7.2
.1 mM EDTA
10mM KCl
5mM Bme
10% glycerol

Q Buffer B

25mM Phos - pH 7.2
1mM EDTA
800mM KCl
5mM Bme
10% glycerol

MT
6/20/95

Pharmacia LKB Biotechnology

Code No. 18-10C

To Page 1

Witnessed & Understood by me,

Date

Invented by

Date

May Long

6/20/95

Recorded by

S. Flynn

6/16/95

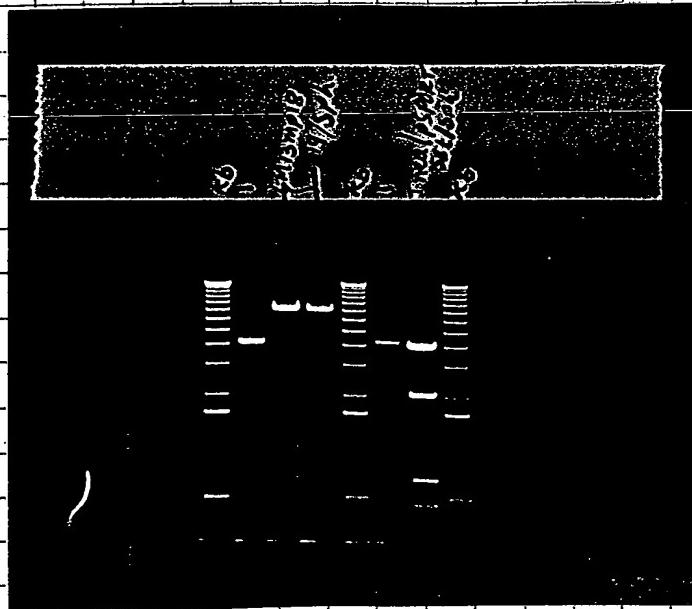
Project No. _____

Book No. _____

ag No. _____

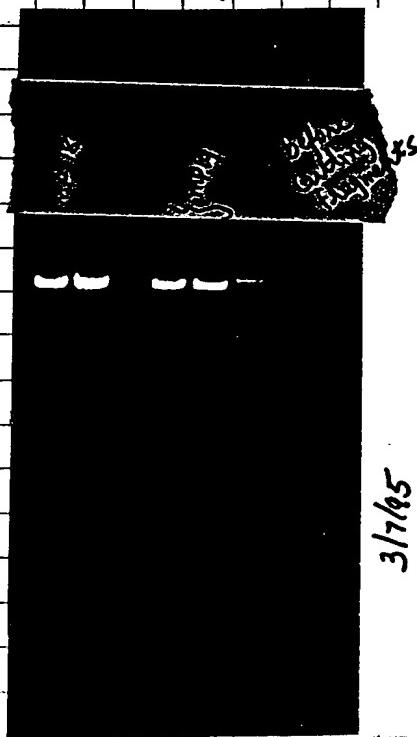
Repeat

3/7/95 TUE

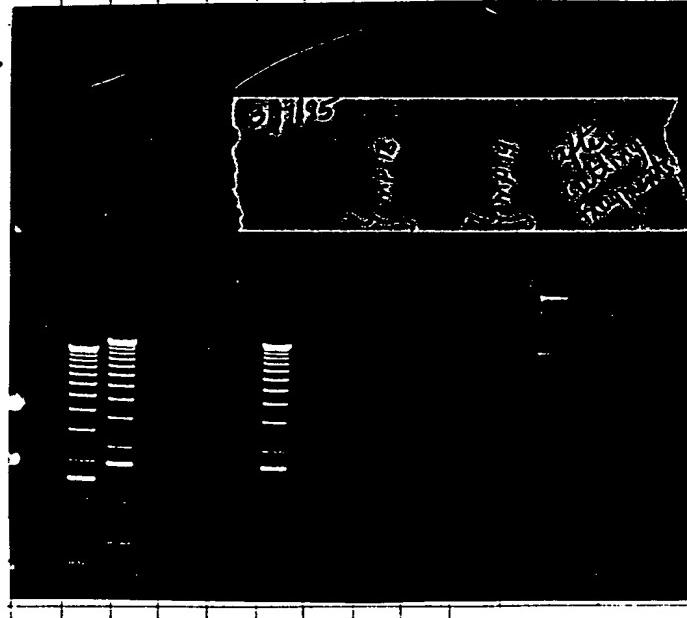


3/7/95

After taking picture or looking @ the gel, M₁₂ & M₁₃ mp 18 and M₁₃ mp 19 is @ the 7.2 Kb which was cut with Sph I. We planned on cutting mp 18 and mp 19 with Sst I. The gel ^{picture} below shows mp 18 & mp 19 before & after cutting the DNA fragments. After cutting the fragment performed Gene CLEAN.



3/7/95



To Page No. _____

is d & Understood by me,

Bo Karp

Dat

4/12/95

Inv nt d by

R cord d by

Date

4/12/95

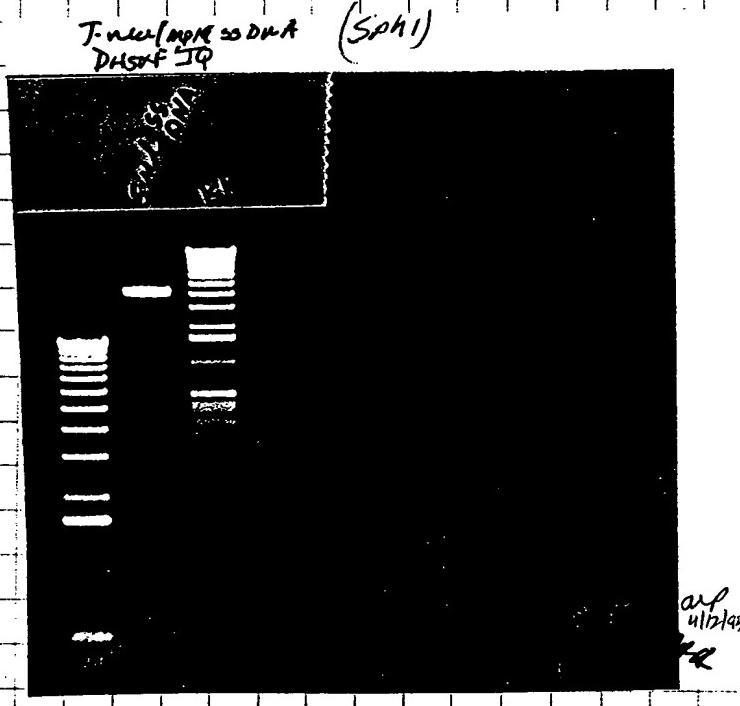
Project No. _____
 Book No. _____ TITLE _____

From Page No. _____

labelled 2 tubes, 1 w/ mp 18, & 1 2nd w/ mp 19.

1. To the DNA w/ agarose gel, added 700.0 μl NaI
 2. Put the tubes @ 52°C heat block to melt agarose. vortexed (constant)
 3. added 5.0 μl glass milk to both tubes - mixed
 4. incubated on ice for 5 min.
 5. Cfg. (quick spin) @ room temp.
 6. discarded supernate, added 500.0 μl New wash buffer
 7. discarded supernate, washed pellet 3x with New wash buffer
 8. after washing 3x, added 14.0 μl dH₂O to the pellet (discarded)
 9. incubated @ 52°C for 5 min.
 10. discarded pellet & saved supernate for ligation.
 (cond this on 3/8/95 wed.)

Purification of m13 ssDNA (T₁ rec 2 kb [Sph I] /mp19) from pg. 1



To Page 1

Witnessed & Understood by me,

John Polans

Date

4/12/95

Inventoried by

Ronald

Date

4/12/95

ag No. _____

cell growth & infection

- Grew an E. coli F' strain to an OD of 0.2-0.4 in 2x YT
- Inoculated 1-2 ml of the cells w/ the phage. (Added 10.0 μl from a liquid phage stock & added to cells)
- Incubated the phage infected cells @ 37°C for 5-7 hours.
- The supernate can now be processed for isolation of ssDNA & the cells can be processed for the isolation of replication form (RF) ds DNA.

Purification of m13 ssDNA

- transferred 1.0 ml culture of infected cell to 4 different eppendorf tubes
- Cfg 4 tubes for 2 min.
- transferred supernate to the new tubes & saved pellet from 1 tube (out of 4 tubes) for isolation of RF DNA
- Spinned the supernate again & transferred the supernate to the new tubes (done to remove any residual cells remained behind)
- passed the supernate through a 0.45 μ filter as to remaining cells (done when performing site-directed mutagenesis)
- added 200.0 μl of 20% PEG + 1.5 M NaCl - vortexed
- Incubated tubes for 15 min @ room temperature (or overnight @ 4°C)
- Cfg. for 10 min in a μcfg. @ room temp.
- discarded supernate & briefly spinned the tubes to remove the residual soln from the side of the tube. (removed as much as possible)
- added 200.0 μl TE - vortexed
- Cfg for 2 min. to remove any residual cell debris.
- Transferred supernate to the new tube. (Added 5.0 μl RNase I to remove any residual nucleic acid from the prep. Benzonase will remove both RNA & DNA very efficiently.)
- added equal volume of phenol / chloroform / isoamyl alcohol. mixed well.
- Cfg for 5.0 min.
- transferred the upper layer to a new tube (BE CAREFUL NOT TO DISTURB WHITE INTERFACE OR REMOVE ANY PHENOL)
- added 200.0 μl NaAc & 600.0 μl EtOH
- Incubated @ -70°C for 5-15 min. (we left @ -70°C overnight) To Page N .

ssed & Understood by me,

Date

Invent d by

Date

JDP/OKCNS

4/12/95

Recorded by

4/12/95

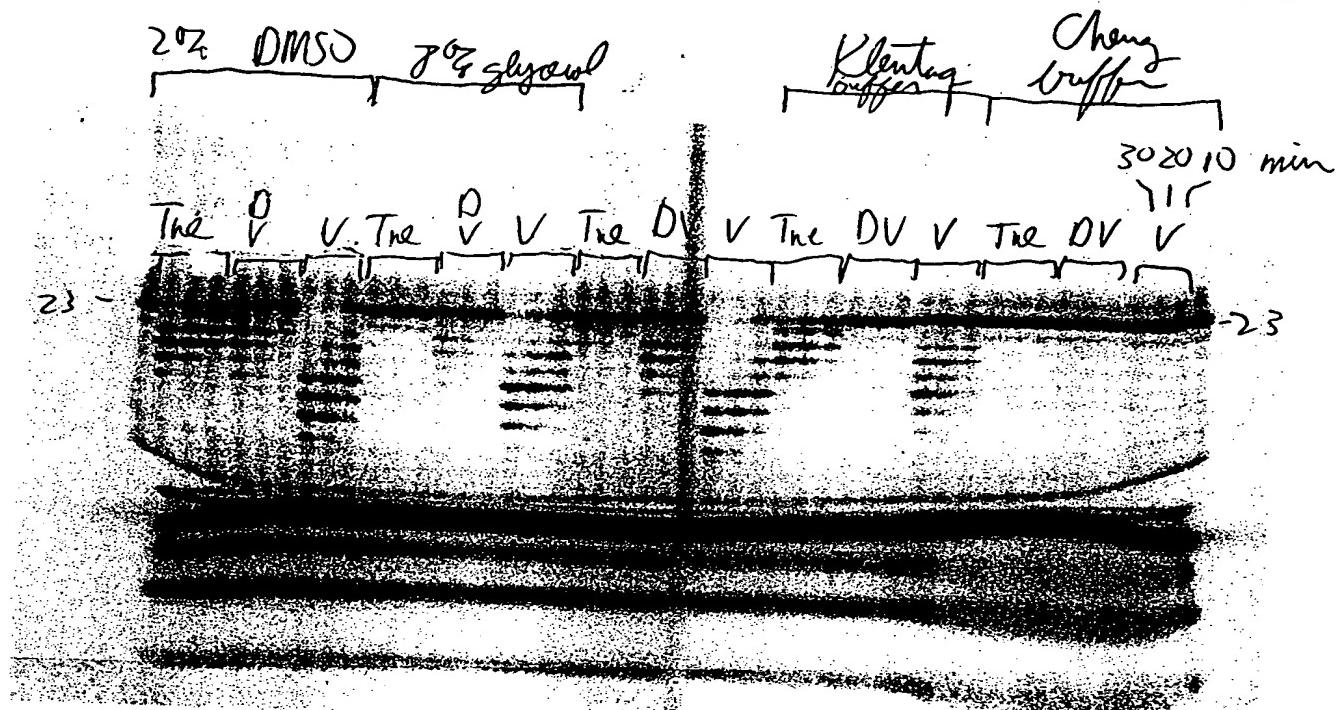
82

Project No. _____

Book No. _____

TITLE _____

From Page No. _____



Result.

Witnessed & Und rstood by me,

Deesara Polap

Date

" 19 94

Invented by

Recorded by

T Pag No.

Date

115-94

Project No. _____

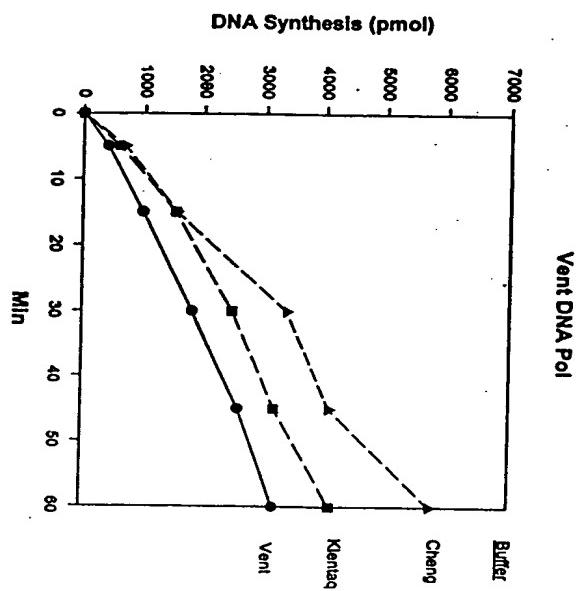
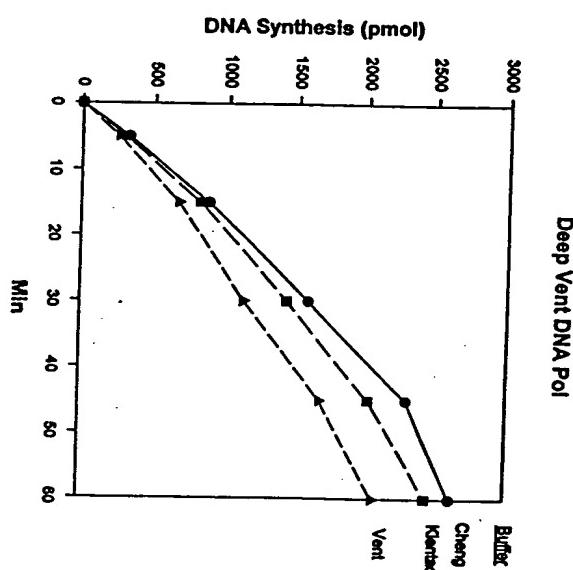
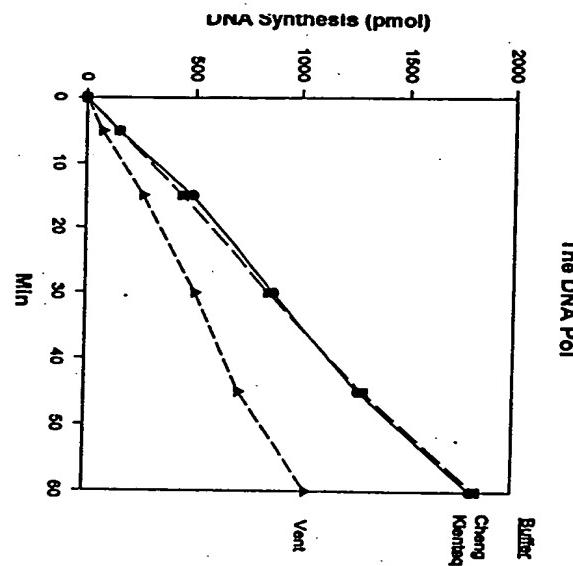
Book No. _____

TITLE _____

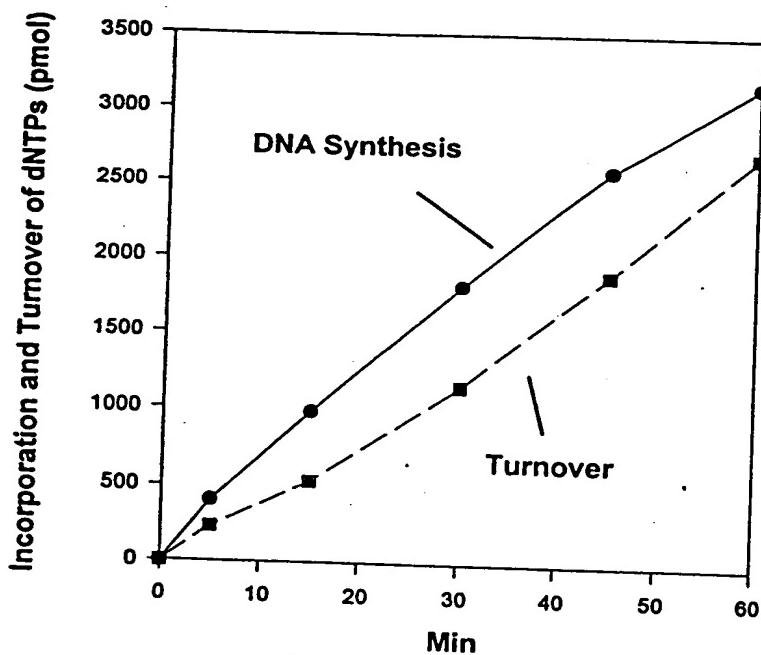
From Page No. _____

In such case, DNA synthesis is lower in
Primer degradation was highest in Vent

got turnover
by DNA synthesis
1. below

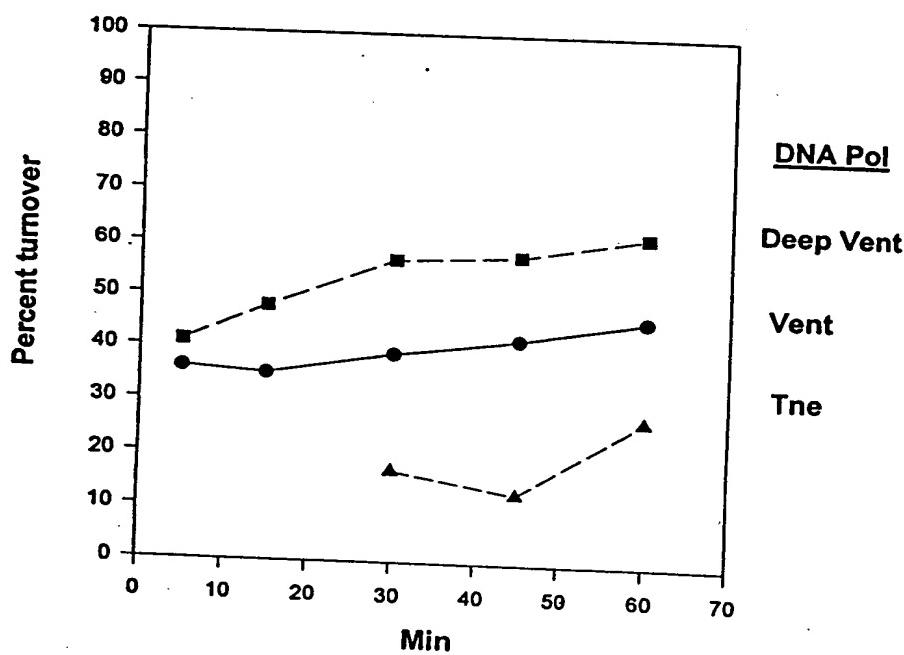


Vent DNA Pol in Vent Buffer



DNA synthesis
and turnover
to dNMP

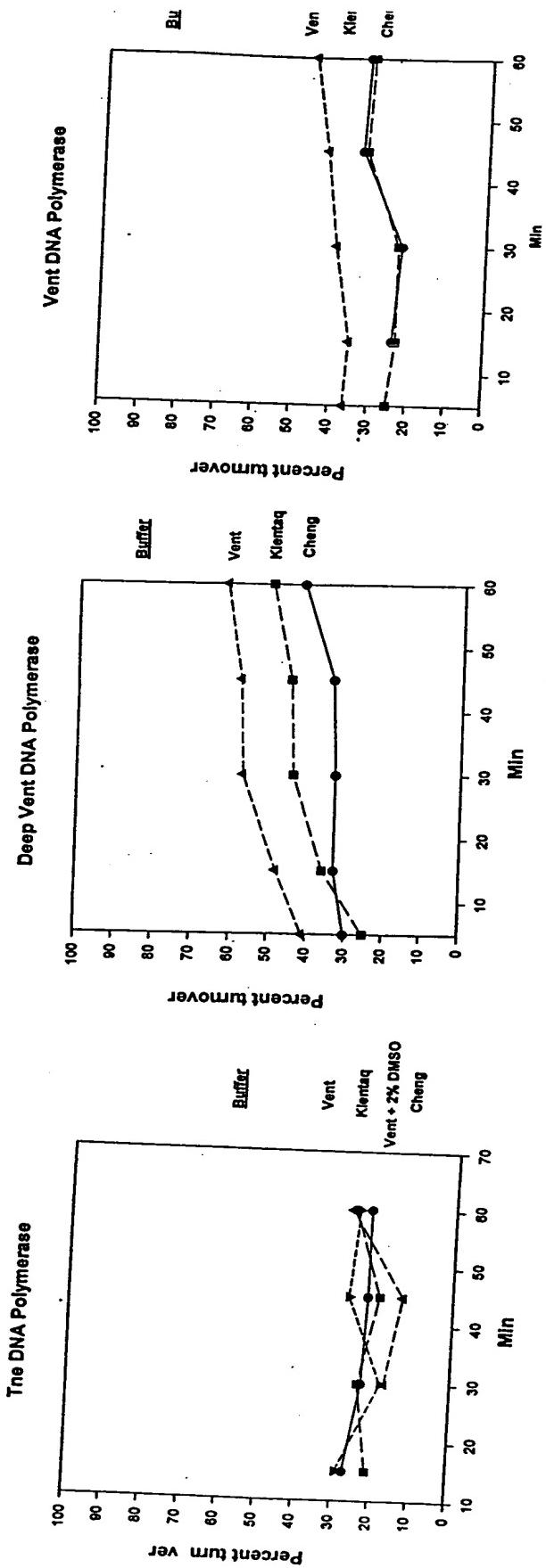
Activity in Vent Buffer



DNA Pol
Deep Vent
Vent
Tne

$$\text{Percent turnover} = \frac{\text{turnover}}{\text{incorporation} + \text{turnover}}$$

Deep Vent has higher turnover than Vent as expected. Tne is $\sim 2\times$ lower than Vent and Deep Vent



Effect of buffer on turnover is not large compared to effect on primer degradation

Understood by me,
Karen Polkup

Date
11/29/94

Invented by
Recorded by

Date
11-5-94

Project No. _____
Book No. _____

TITLE

Turnover for Vent, Deep Vent
(follow P. 61, 7)

From Page No. _____

H₂O
 5 x Cheung buffer
 10 x Klentab
 10 x Vent buffer
 Tag storage buffer
 3.7 mg/ml activated
 DNA

DATGTTTP 10mM each
³²PdATP 10mCi/ml
 Mg(OAc)₂ 50 mM
 MgSO₄ 100mM
 DMSO 100%

(A)
 399 456
 133

(B)
 484 464
 66.7

(C)
 489 4
 66.7

* Tag storage buffer
 Vent 0.08 u/l
 Deep Vent .08 u/l
 Tris 0.08u/l

0.065 ml 0.633 ml 0.633.680 use 1
 (1) 195 (2) 195 (3) 195 (4) 190 (5) 190 (6) 190 (7) 190 (8) 190
 4 4 - 4 4 4 4 4

remove 15 uL to 5 uL 0.2 M EDTA → spot 15 uL on G.
 and remove 5 uL to 5 uL kill solution (20 μmole/l DA
 100 mM EDTA) at 9

0 5 15, 30 45, 60 min
 spot 2 uL on PEI
 resolve in 1M LiCl

* dilutions of pulse
 same as P.J.

Results : see graph on PJ

Witnessed & Understood by me,

Devaraj Bolan

Date

11/29/94

Invented by

R cord dby

Dat

11-9-94

To Pag

ig N . (D)

14.4 ✓

✓

66.7 20

✓

 $1 \mu\text{l} / 100 \mu\text{l PCR} \Rightarrow Cf = 0.005\% \text{ Tween 20/NP40}$ \therefore this makes up for no T_P_L
here - its present in Joe's
long PCR Rxn.

→ 27 ✓

✓

 $(C_p = 50 \mu\text{M each})$

✓

 $(220 \times 10^6 \text{ total cpm})$

✓

 $(1.2 \text{ mM Mg(OAc)}_2$

✓

 $(1.2 \text{ mM Mg SO}_4 \text{ in Klenow buffer}$

✓

 $2 \text{ mM Mg SO}_4 \text{ in } 1X \text{ Vent buffer})$ — 4 μl ✓ $Cf =$ $(2\% \text{ OMSO})$

(10)

17.4 ✓

✓

 $(0.4 \text{ mits total of each pool})$

4

,

To Page N .

Ied & Understood by me,

Sarah Polcari

Date

11/29/94

Invent d by

Recorded by

Date

11-9-94

From	To	Chq	DN	TW	Chq	DN	TW
01	543.00				61	269.00	
02	650.00	110	(14)		62	7412.00	71
03	1014.00	486	24		63	16953.00	9553
04	1485.00	971	22		64	36825.00	3374
05	2627.00	2148	34		65	44610.00	4087
06	3187.00	2725	32		66	62771.00	5752
07	525.00	BKHD			67	241.00	
08	662.00	141	30		68	3518.00	322
09	948.00	436	33		69	9506.00	871
10	1271.00	769	33		70	17320.00	1587
11	1677.00	1188	34		71	25050.00	2296
12	2340.00	1871	42		72	28643.00	2625
13	624.00	BKHD			73	324.00	
14	694.00	72	(32)		74	1974.00	15
15	796.00	177	27		75	5340.00	489
16	880.00	264	23		76	9478.00	869
17	976.00	363	22		77	13880.00	1272
18	1110.00	501	22		78	19753.00	1810
19	805.00	BKHD	775 AVE		79	321.00	
20	977.00	192	25		80	8826.00	582
21	1409.00	467	23		81	23029.00	1533
22	1803.00	762	23		82	37324.00	2485
23	2832.00	1135	32		83	47661.00	5173
24	3299.00	1883	31		84	61758.00	4112
25	774.00	BKHD			85	404.00	
26	918.00	99	25		86	4493.00	299
27	1406.00	465	36		87	12238.00	815
28	2277.00	1118	44		88	21497.00	1431
29	2989.00	161	41		89	30491.00	2030
30	4085.00	2472	50		90	36800.00	2450
31	777.00	BKHD			91	214.00	
32	813.00	21	(12)		92	2257.00	150
33	947.00	121	21		93	6671.00	444
34	1136.00	263	24		94	12685.00	845
35	1204.00	314	19		95	19429.00	1294
36	1631.00	633	26		96	27534.00	1855
37	919.00	BKHD	922 ave		97	239.00	
38	1284.00	231	36		98	7128.00	404
39	1754.00	530	35		99	17335.00	881
40	2728.00	1150	39		100	32171.00	1821
41	3910.00	1903	42		101	45795.00	2592
42	5168.00	2704	46		102	56065.00	3174
43	924.00	BKHD			103	318.00	
44	1205.00	180	41		104	4474.00	253
45	1892.00	617	47		105	11839.00	670
46	3234.00	1472	57		106	19756.00	1119
47	4572.00	2325	58		107	29674.00	1470
48	6365.00	3467	62		108	36540.00	2069
49	863.00	BKHD			109	261.00	
50	901.00	—	(7)		110	1566.00	74
51	953.00	20	(7)		111	4647.00	263
52	1083.00	103	17		112	8879.00	503
53	1085.00	103	13		113	12496.00	707
54	1529.00	386	27		114	18327.00	1037
55	984.00	BKHD			115	295.00	
56	891.00	—			116	1709.00	80
57	1067.00	92	29		117	4261.00	224
58	1086.00	104	18		118	8343.00	9929
59	1336.00	364	—		119	12504.00	708
60	1467.00	347	25		120	18443.00	1040

With

Invented by

11/29/94

Record d by

11/9/94

Deborah Polans

g N. _____

JAMP BKGD⁰

1. Chevy mix = 564 ave
2. Klentay mix = 785
3. Vent mix = 922

spot

Chevy

$$75821 \text{ cpm} \left(\frac{5 \text{ ml Rxn vol}}{2 \text{ ml spotted}} \right) \left(\frac{200}{175} \right) \left(\frac{1}{2500 \text{ pm}} \right) \left(\frac{1}{4} \right) = 194 \frac{\text{CPM}}{\text{pm}}$$

DAT

194 pm/nt
267 cpm/turnover

Klentay

Vent

314 cpm/pmt

$$\text{pmol incorp} = \frac{(200 \text{ pm})}{(200 \text{ pm/km})}$$

$$\frac{\text{CPM}}{\text{CPM/pmol}} \frac{(200)(20)}{(15)(15)}$$

$$\text{pmol turnover} = \frac{(200 \text{ pm/km})}{(200 \text{ pm})}$$

$$\frac{\text{CPM} - \text{BKGD}}{\text{CPM/pmol}} \frac{(200)(10)}{(5)(2)}$$

$$\% \text{ turnover} = \frac{\text{pmol turnover}}{\text{pmol turnover} + \text{pmol incorp}}$$

21 75821.00
22 104512.00

To Page No. _____

& Understood by me,

Date

Inv nted by.

cinda Polany

11/29/94

R corded by

Date

11-10-94

PAGES 88-89 OF NOTEBOOK WERE BLANK

AT (all) carried Project No. _____
90 out at Frederick Book No. _____

TITLE

Repeat unit assay QC for rTAQ
lot # EKBT1 done on p. 61 except

From Page N. _____

Amplifast lot # 9957 for control

lot EKBT1 is ~ 4.01 u/ml based on p. 61

1. starting dilutions of EKBT1

1:80 (estimate Cf = 5 uA)

lot EKBT1

5 uL

Taq storage

385 uL

actual is 4.03 u/ml

Vf = 400 uL

1:160 (estimate Cf = 2.5

5 uL

385 uL

actual is
2.01 uL

Vf = 600 uL

2. 1/600 dilutions

serial dilution # 1-6 7-12 13-18 19-24 25-30 31-36 37-42 43-48 49-54

1:80 dil.

I II III IV V VI A1 A2 A3

1:160 dil

3 3 3

3 3 3

Amplifast 5 uA

Vortex 5 s

lot # 1

A1 A2 A3

dilution buffer

179 uL

use from h
20 and 40 mi

Vf = 2000 uL
1800 uL

dilute I - A3 as shown for I below:

3. Serial dilutions

dilution
buffer

dilute I - III and assay

then dilute IV - VI and assay

then dilute A1 - A3 and assay

serial dilutions # 1 100 uL 7300 uL
2 100 uL 7300 uL
3 100 uL 7300 uL
4 100 uL 7300 uL
5 100 uL 7300 uL
6 1mL of I 7300 uL

SA I - III = 45 uL assay mix + 5 uL dil buffer, do same for IV - VI

spot 4x 5 uL on 6FC in your aquasol

Blank is 45 uL assay mix + 5 uL dil buffer → spot on 6FC along with other

To Pag N

Witnessed & Understood by me,

Deepti Rana

Date

1/6/95

Invented by

Deepti Rana

Date

11-15-94

sg N _____

55-57 = Blanks for I-II, IV-VI and A1-A3 respectively

58-61 = SA for I III

62-65 = SA for IV-VI

Results:

using ampholyte lot #9957 here gives a unit value of ~~320 u/l~~ 323.4 u/l (compared to 401 u/l (found on P61, 10-1-94))

To Page No. _____

Signed & Understood by me,

Suzanne Polking

Date

1/6/95

Invented by


R corded by

Date

10-15-94

Project No. _____
 Book No. _____

New dilution of EKBT1 to
 TITLE 10 u/l for Larry Martz

From Page No. _____

* will use old unit value of 401 u/l No can make
 old dilution & carry over in October
 (see P 91 where final unit determination for EKBT1
 is 323.4 units / μ l)

Calibrated P20 (P0)
 its exactly 10 μ g for
 for P1000 (P0185) we
 37 μ l which goes
 391 μ g

Tag storage buffer 391 μ l

Tag lot (# EKBT1)
 1 ("401" u/ μ l)

10 μ l

* see above

$V_F = 401 \mu\text{l}$ (10 μg units)

1. Bring Tag storage buffer to room Temp.
2. Bring small aliquot (aliquot of EKBT1 (main stock)) to room temp.
3. deliver 10 μ l Tag into 391 μ l storage buffer, rinse ~10 times (i.e. triturate)
4. mix with P1000 to get in all storage buffer
5. vortex 5 sec
6. mix end over end in cold room 2 hr

T Page No. _____

Witnessed & Underst od by m ,

Date

Invented by

Date

Deborah Roberts

6/95

Recorded by

11-30-94

Log No. _____

Equidetec 2mL AccSO m w/ Q buffer A - 6/17
dilute load of 3

Load on 0.5 mL/min - soon Sensitivity - .05

Wash to base w/ Buffer A - collect F.T. - 0.1mL/min

Program - ① 5mL wash w/ Q Buffer A @ .5mL/min
② 20mL linear gradient 0 → 100% Q Buffer R
③ 5mL was w/ Q Buffer B @ .5mL/min
Collect 500μl fractions -

Gamma Activity Assay -

μl of premix aliquotted to prelabelled eppendorfs -
incubate @ 74°C for 10 min quench w/
10 μl of .5M EDTA - spot 3 20μl on 6PTC
filters - TCA was a
1x 10% TCA + 1% Pi @ 5'
3x 5% TCA + @ 5'
2x EtoH
dry + count on LSF-Econost

Pool 2u-35 dialyze o/N (over weekend) - against
Taq storage buffer (No detergents) -

1 - Remove ~ 1.8mL from dialysis - store in 2mL eppen
Hot PINK - -20°C

To Page No. _____

ssed & Underst od by me,

Date

Invent d by

Date

May Long

6/20/95

Recorded by

S. Flynn

6/19/95

Q65D - T85D his - 2mL column

Project No. _____
Book No. _____

147

ag N.

* FY-1

06/16

sump + column w/ .5N NaOH -

wash extensively w/ H₂O

equilibrate w/ Q65D-Buffer A - D. 146 -

Load ~ 3.5mL of Heparin pool of FY-1 @ .5ml/ml

Wash with QBuffer A until base line is reached -

Gradient - 20mL linear gradient 0-100% Qbuffer B
(@ .5mL/min collect - 5mL fractions)

Wash w/ 10mL of 100% Qbuffer B - collect
.5mL/min fractions -

Fraction collector - started then stopped after
fraction 10/11 - Did not realize until
gradient was finished - lost entire elution
~~effluent~~ to waste! Could have tried to
Save however I believe I washed the port
w/ .2N NaOH & in the same waste container

Fraction collector stopped b/c outside of rack was "dirty"
and was slipping - Must be sure outside plastic is
clean!

Plan to proceed with 3'-5' exo mutant - Run b column
with 3M KCl - wash w/ H₂O equilibrate w/
Q65D Buffer A D. 146.

To Page No. _____

is d & Understood by me,

Date

Inv nted by

Date

May Longo

9/20/85

R cord d by

OC/16/95

Project No. _____
Book No. _____

TITLE

Q650M - 3'5' EXO minus - T

From Page No. ____

41

0-800mM KCl in ② pH 7.2

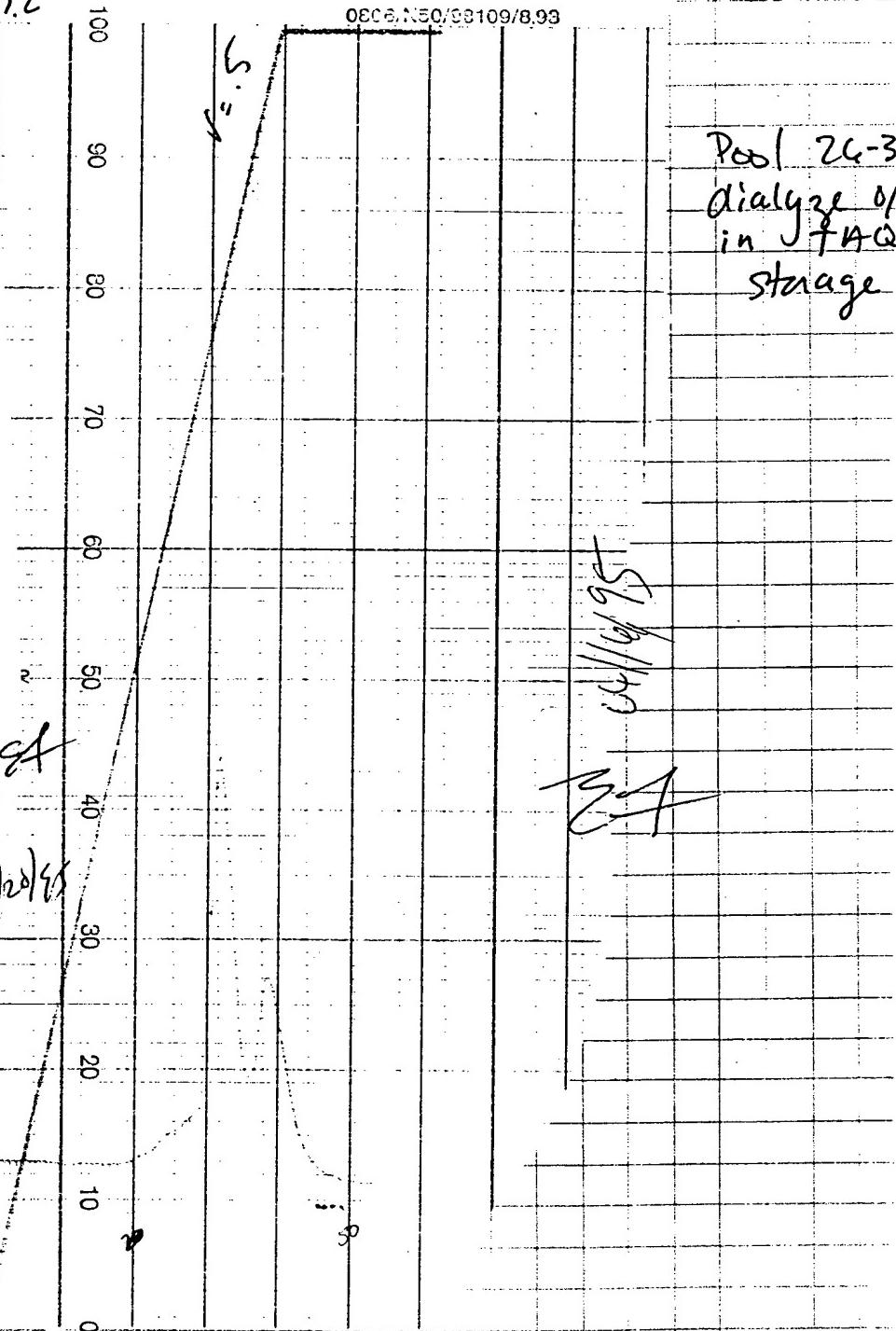
SAM CPM1

fraktion
 1 20 188.00
 2 22 138.00
 3 24 180.00
 4 26 874.00
 5 28 830.00
 6 30 748.00
 7 32 1174.00
 8 34 912.00
 9 36 556.00
 10 38 590.00
 11 40 340.00
 12 42 326.00
 13 44 370.00
 14 46 266.00
 15 48 298.00
 16 50 186.00
 17 9928.00
 18 198.00

pool

load

pool 26-35



Witnessed & Understood by me,

Date

Invented by

Date

Allen Jones

6/20/95

Recorded by

6/12/95

Page No. _____

Purpose: Verification of pg 124 - 125

Amplified linearized puc / xmr 1 using 2 different new sets of primers

36

37

38

39

Tried with Tag and Tag + D.V.

Incubated Mg 1.5, 2.0, 2.5, 3.0 mM cycling 94° 30" 1

200 µg dNTP

1.4 mM primer

produce = 1275 bp.

1 U of enzyme - Tag

25 µg template

(94°, 30 s) 30

65°, 1'

Resuspend 10x of each : Tag 1 * 3 Tag + DV 1 * 3

11 1 * 2

11 1 * 2

11.20 338

330 µl

1.5 2 2.5 3 mM

0.5 buffer 50

7.5 10 12.5 15

dNTP 10

42.5 40 37.5 35

Mg -

50

primer 1 20

2 20

Template 1.0

enzyme 2 10 µl Tag + DV

450

45 µl / Rx added 5 µl of Mg dif. conc.

To Page N

Signed & Understood by me,

Date

Inventoried by

Date

12/9/94

Recorded by

12/9/94

R. Slaeaman

Project No. _____

128 Tolu

Book No. _____

TITLE _____

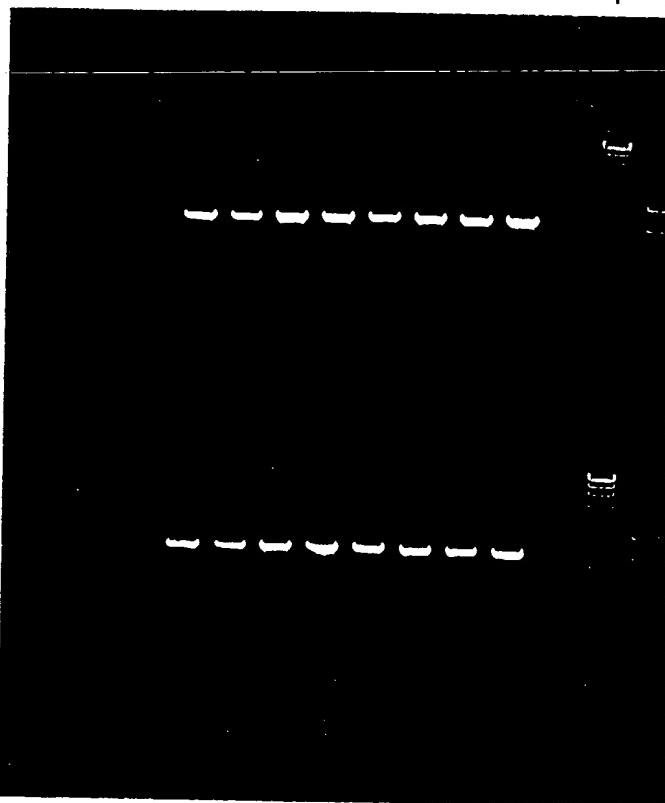
From Page No. _____

Tag

0 1.5 2 2.5 3 mmg Mg₂

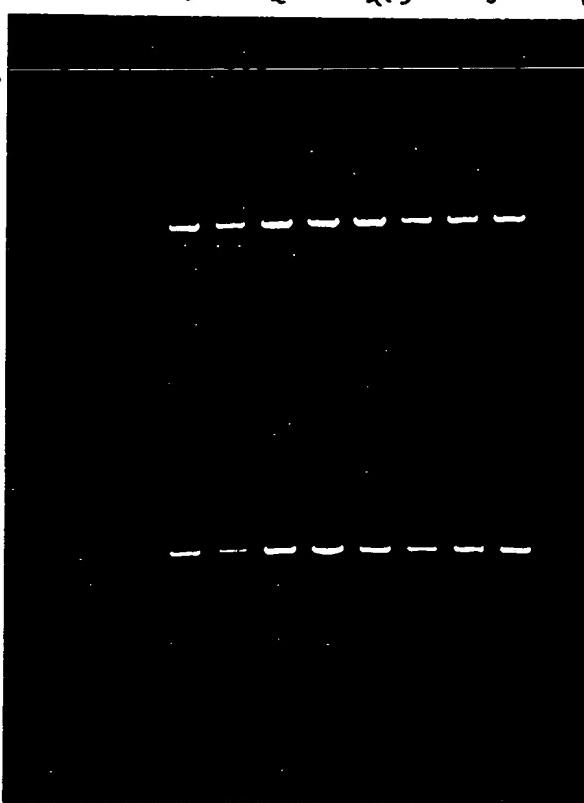
Tag + DV

0 1.5 2 2.5 3 mmg

2836
✓
37

X3

X2



2838x89

in 1275 bp product.

Both primers set work with Tag as well as Tag + DV but if sequencing still has to be gel purified great range of Mg²⁺ tolerance.

Pooled (1) Tag 1.5 mM Rx Separately) with X 3
(1) 2.0 set of primer

(2) Tag + DV 1.5 mM
(2) 2.0

✓ and phenol in ethanol 1/1
To Page

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

Dr. S. S. Varma

12/18/84

'ag N .

mixed Rx from two tubes. (duplicate of same) together is 30 µl + made up the volume to 100 µl 30 µl

added equal amounts of phenol: chloroform: ice amalgalated removed the aqueous phase after a spin of 5' - phenol extracted again.

added 0.5 volume of 7M ammonium acetate and 1 ml of ethanol, added also a ml of Dextran T 500.

Spin down, remove ethanol, washed the pellet with 70% ethanol, spun down, removed the sept.

spins again to remove the residual ethanol
pellet wash. Vacuum dried 5'.

suspended in 17 ml of medium 2 ml for gel

for 15 ml added

10.5 11 H₂O

3.0 " 10 x buffer

1.0 " AgI $\frac{1}{11}$ (70/1x)

0.5 .. Ääl- ii (240/j.s.)

3.0 ml uncoated at 37° 2 hrs.

phenol extracted product seems to be around $\sim 150 - 200 \text{ mg/g}$ /xx
 $\sim 495 \text{ mg/g}$ T_x

To Page No..

~~Used & Understood by me~~

Dat

Invent'd by

Det

12/1987

Recorded by

12/18/84

K. Sitarman

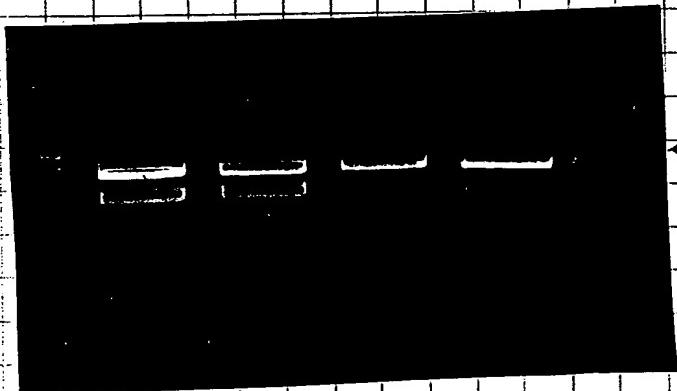
Project No. _____

Book No. _____

TITLE _____

From Page No. _____

- 15 μ l of left over phage chloroform extracted + ethanol precipitated insert + was cut with Pst I and Apa I in NEB buffer 4 for 2 hrs at 37°.
- Run on 1% gel and transferred to DEAE paper and eluted the fragment in high salt buffer, over the cIM NaCl, 0.1M Tris pH 8.0, 5 mM EDTA
- spun down the ethenol supernatant added 50 μ l mmq p. + centrifuge, pellet the ethanol, ethanol applied in 150 μ l → 500 μ l via the presence of 1 μ l of desalting T-400.
- left at 70°, 2 1/2 hrs, resuspended in 15 μ l of the ethanol wash, in 70°.



→ extracted, to serve as insert!

$$\text{loaded} \approx 75 \text{ ng} \times 15 \mu\text{l} = 1125 \text{ ng} (1275 \text{ bp}) \\ = 772 \text{ ng} (875 \text{ bp})$$

$$\approx 50\% \text{ recovery} = \approx 386 \text{ ng} / 15 \mu\text{l} \\ = \approx 25 \text{ ng} / \mu\text{l}$$

To Page _____

Witnessed & Understood by me,

Date

Initiated by

Date

12/19/84

Recorded by

12/12/84

K. Stearns

28

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

3/8/95 wed.

- centrifuged the samples for 10-15 min.
- discarded the supernate & rinsed the pellet w/ 70% EtOH
- dried the pellet @ 55°C heat block or @ room temperature
- dissolved the DNA in 50.0 μl TE.

SDS Rxn

3/8/95 wed.

Annealing Rxn.

+ Primers (2899)	- Primers (2899)
------------------	------------------

H ₂ O -	3.0 μl	4.0 μl
--------------------	--------	--------

5X Buffer	2.0 μl	2.0 μl
-----------	--------	--------

μg. 2 ⁶ SS DNA	4.0 μl	4.0 μl
---------------------------	--------	--------

(200ng/μl) Klenow	1.0 μl	—
----------------------	--------	---

T _V	10.0 μl	10.0 μl
----------------	---------	---------

Incubated @ 70°C - 75°C for 2 min. (to eliminate non-sp. bsr.)
 " @ 37°C - 40°C for 2 min.

Synthesis Rxn

→ Annealing Rxn - 10.0 μl.

5mM 10X buffer - 2.0 μl

H₂O - 6.0 μl

T_V/T₇ DNA poly - 1.0 μl

T_V DNA ligation - 1.0 μl

T_V - 20.0 μl.

Incubated @ 37°C for 10 min.

Synthesis Rxn - 2.5 μl

TE - 8.0 μl

Loading dye - 1.0 μl

- ran the sample on the gel
- the picture on the next page # 29.

To Page No. _____

Witness d & Und rsto d by m ,

Date

Inv nted by

Dat

John Doe

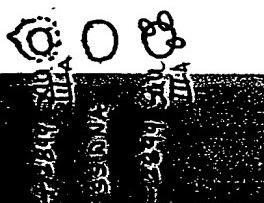
4/12/95

R c rded by

John Doe

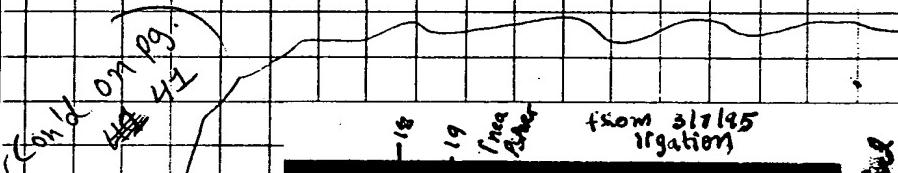
4/12/95

'age N. —



(con'd from pg. 28)

+2899 (w/ primer) oligo forms a ds DNA ∴
+2899 fragment looks brighter because
Et-Bromide binds to it better. -2899
Primer binds but ^{cooperatively} it does not hold strongly
∴ the DNA fragment looks fainter or
light, less Et-Bromide is able to bind.



Ligation from 3/7/95 (pg. 26)

<u>H₂O</u>	-	8.0 mL
<u>5X buffer</u>	-	4.0 mL
<u>Imp. IB</u>	-	2.0 mL
<u>insert</u>	-	4.0 mL
<u>ligation</u>	-	2.0 mL
<u>TV</u>	-	20.0 mL

H ₂ O	-	8.0 ml
5x buffer	-	4.0 ml
(vector) mp 19	-	2.0 ml
insert	-	4.0 ml
<u>ligation</u>	-	2.0 ml
T.V.	=	20.0 ml

- Incubated both samples for 1 hour @ room temp.

ran mp 18 on 3/10
(used DNA from
3/10/95 again on
3/15/95 19-32)

00.0 μl Competent cells }
3.0 μl DNA } Xfection
cells.

xfection

3 10% mp 18 / mp 19
 90% mp 18 / mp 19
 Control

To Page No..

As I See & Understood by me,

Date

Invented by

Date

12/90

Recorded by

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

- 10% mp 18 / mp 19

added (4.0 mL 2X YT TOP Agar
 100.0 μ l X-Gal 4%

5.0 μ l IPTG 200 mM (inducer = repressor gives tighter affinity)
 60.0 μ l lawn cells

10.0 μ l infection cells (after heat shock for 35 sec.)

- 90% mp 18 & mp 19.

Same way as 10%

- Control

100.0 μ l X-Gal5.0 μ l IPTG60.0 μ l lawn cells.

100% no 3:45 12:00

T Pag N

Witnessed & Understood by me,

Date

4/12/95

Inv nt d by

Recorded by

Dat

4/12/95

miniprep and digest of
pUC 19 PCR products

Project N _____
Book No. _____

Exhibit 18
Appl. No. 09/558,421

93

Log No. _____

11-7-94 received plates from Kala S. for pUC19 PCR with Taz + Deep Vent and no Mn⁺⁺ or dNTP bias.

picked 20 white colonies and 2 Blue for 2 ml LB+100 μg/μl overnights at 30°C.

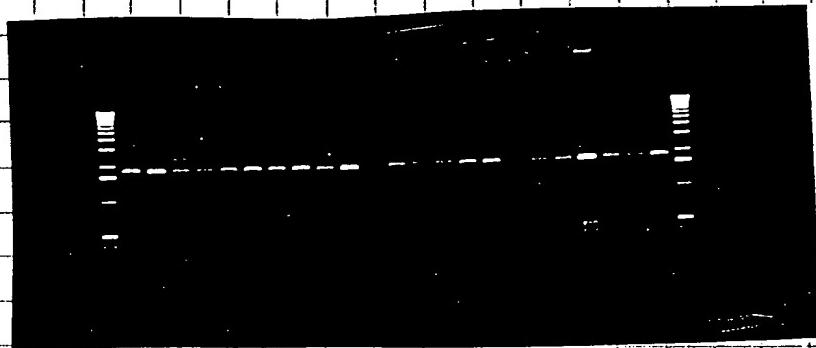
12-9-94

minipreps as per (p 41, 4)
using 0.5 ml cells.

resuspended DNA in 50 μl TE + RNase

digest each (#21, 22 ^{are from} _{all from} 2 blue colonies)
as follows

Buffer 4 (10x)	11.6	✓ ✓ ✓	569	7
	2	✓ ✓ ✓	94	
R1 10 ^{aM} / ₁	5.5	✓ ✓ ✓	23.5	cocktail for 47 Rxn
mH1 10 ^{aM} / ₁	0.5	✓ -	are 15 μl Rxn	5 μl DNA
T II 24 ^{aM} / ₁	0.1	✓ ✓ ✓	4.7	
I III 7 ^{aM} / ₁	0.3	✓ ✓	14.1	
	20 μl		37°C	2 hours



2 loc 2 gels 2 bands

To Page No. _____

signed & Understood by me ,

Date

Invented by

Leanne Bolens

17

6/95

Recorded by

Date - 7-94

12-9-94

150

Project No. _____

Book No. _____

TITLE The - 3' 8' 11' 14' 15' 16' 17' 18' 19' 20' minos -
36 S1

From Page No. _____

B Enzyme titration -

Thaw - 2 vials of Tag mix - add 22 μ l to the total
of 2 dCTP - 32P-

aliquot 4 μ l to prelabelled eppendorf's - on ice -
add 1, 2, 4 μ l of diluted enzyme - incubate 10'@
74°C in a heat wakry block - quench w/ 10 μ l of 5%
EDTA - spot 30 μ l on GF/C - wash

1X 10% TCA 1% Pi
3X 5% TCA
2X EtOH

Dry and count in LSC
under heat lamp.

SAM	CPM1
1	1864.00
2	2938.00
3	2940.00
4	940.00
5	1658.00
6	2606.00
7	404.00
8	320.00
9	732.00
10	152.00
11	306.00
12	384.00
13	126.00
14	238.00
15	326.00
16	118.00
17	106.00
18	134.00
19	112220.00

07/29/95

To Page #

Witnessed & Understood by me,

Date

Invented by

Date

May Longo

07/29/95

Recorded by

07/27/95

ge N . _____

4/27

AM CPM1

1	6084.00	.063
2	10302.00	
3	8286.00	
4	3506.00	7.72
5	4842.00	.050
6	5272.00	
7	1370.00	8.5
8	1842.00	5.7
9	3392.00	5.27
10	182.00	
11	85826.00	
12	92658.00	
13	92494.00	

SB 1/27/95

nl - Pool - .075 u/ml \rightarrow 150 Units total -

TIME AVG H#

0.50	35.0
0.50	36.0
0.50	40.0
0.50	48.0
0.50	38.0
0.50	42.0
0.50	52.0
0.50	40.0
0.50	40.0
0.50	43.0
0.50	42.0
0.50	44.0
0.50	49.0
0.50	44.0
0.50	42.0
0.50	54.0
0.50	53.0
0.50	46.0
0.50	34.0

.08 u/ml

.08 u/ml

.063

.08

.071

.055

ERR

SA = 70.1 cpm / pmol
7.01 x 10⁵ cpm / nmol

SA = 70.1 cpm / pmol

7.01 x 10⁵ cpm / nmolMNF
4/29/95

To Page No. _____

Read & Understood by me,

Date

Invent d by

Date

Mary Lugo

4/29/95

Recorded by

4/27

.15

ge N .

purpose: To ligate the purified Vector + insect and transposon with appropriate controls.

gatium Rx:

Testid

Vector ~ 100ng/ μ l	=	1 μ l	1	1 "	1 Vector alone
insect ~ 25ng/ μ l	=	4 μ l	2	" " + real apo	2 " " + apo
5x buffer	=	4			
Lugene T4		1			
H ₂ O		10			
		20 μ l	3	hrs.	3 - Tag 1.5
			4	" "	4 " "
			5	" DV 1.5	5 " DV 1.5
			6	" "	6 " "

transformation using DH5 α Max eff. cells.

total produced used 2.5 μ l of lig chain mix / transformation 50 μ l cells initial volume after adding SOC ~ 500 μ l

split 25, 50 & 600 μ l of each

control diluted to 1:10 and plated 25, 50 & 100 μ l. normal transformation efficiency Vector only - few blues because of the contamination $\sim 1.5 \times 10^9$ no white

Vector + insect - ligated w/o any purification, lots + lots of colonies transformation quite efficient

Tag + DV } 2 mM - didn't work

Tag alone }

Mg

Tag " } 1.5 mM

Tag + DV } Mg

Very few colonies in 25 μ l & 50 μ l
50 μ l slightly better. No deep blue
for ^{blue} than Tag alone, however,
are too few to make a call

- all purified gave low efficiency of transformation compared to un - vector + insect

Page No.

ed & Understood by m .

Date

Invented by

Date

12/12/94

Recorded by

12/13/94

R. Subramaniam

uge No. _____

3/14/95 TUE

Procedure: miniprep

1.0 ml culture of T-neo/Imp 19 grown for 5 hours @ 37°C fin
 6 different glass tubes
 transferred 1 ml cell to the 6 different labelled eppendorf tubes.
 cfg all 6 tubes for 2 min. @ room temp.
 removed supernate & saved in different tubes
 added 100 μl SI mixed well
 added 200 μl S2. put the tubes on ice (mixed by inverting)
 added 150 μl 7.5 M NH₄OAc.
 incubated on ice for 5 min.
 cfg for 7 min. @ room temp (4°C) ^{NOTE: cfg in 4°C room was taken away for superci: used @ RT}
 transferred supernate (400.0 μl) to the new 6 labelled tubes
 added 800 μl of EtOH to the 400 μl of supernate (mixed well)
 incubated @ -70°C for 30 min.
 cfg for 2 min. @ room temp (discarded supernate)
 rinsed w/ 70% EtOH (removed supernate)
 added 50.0 μl TE to the pellet

H ₂ O - 7.0 μl	x 6	tubes	= 42.0 μl
buffer - 2.0 μl	x 6		= 12.0 μl
Eco 47III - 1.0 μl	x 6		= 6.0 μl
		TV	60.0 μl

added 10.0 μl DNA to each 6 tubes.

^{the} map is on next page # 32. Fragments on all 6 tubes are still present, y haven't gone into the mutant.

• I tried miniprep again next day
(started)



d & Understood by me,

Date

Invented by

Date

Polkans

4/12/95

Recorded by

4/12/95

miniprep and digest of
pUC 19' PCR products

Exhibit 19
Appl. No. 09/558,421
Pr j ct N _____
B ok N _____

93

Ag No. 12-9-94 received plates from Raka S. for pUC19 PCR with Taz + Deep Vent and no Mn⁺⁺ or DNTP bias.
picked 20 white colonies and 2 Blue for 2 ml LB+100µA overnights at 30°C.

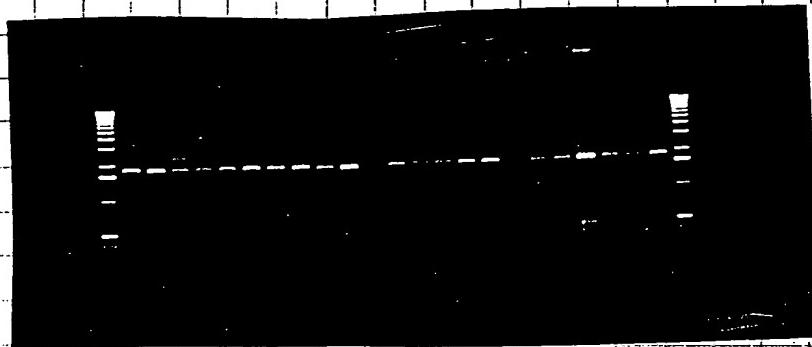
12-9-94

minipreps as per (P 41, 4)
using 0.5 ml cells.

resuspended DNA in 50 µl TE + RNase

Digest each (#21, 22 ^{and from} the 2 blue colonies) as follows (Tube 23 is 0.2 µl of 0.8 µg pUC19)

Buffer 4 (10x)	11.6	✓ ✓ ✓	569
Taq	2	✓ ✓ ✓	94
RL 10 µg/ml	5.5	✓ ✓ ✓	23.5
m H 10 µg/ml	0.5	✓ -	use 15 µl rxn 5 µl DNA
+ II 24 µg/ml	0.1	✓ ✓ ✓	4.7
III 7 µg/ml	0.3	✓ ✓ ✓	14.1
	20 µl		37°C, 2 hours



3 loc 2 gels 2 bands

Result:
all are
digested
for 2

To Pag No.

seed & Und rstood by m ,

Date

Invented by

Date - 7-94

Received By

1/6/95

Recorded by

12-9-94

Project No. _____

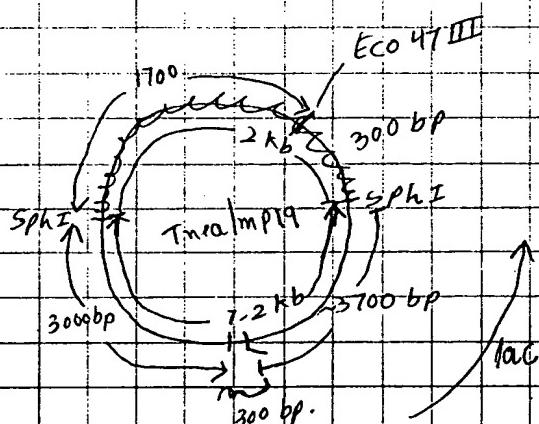
Book No. _____

TITLE _____

32

From Page No. _____

3/15/95 Wed



Eco 47 III

parent

~ 8.9 kb

0.3 kb → (most probably won't see fragment because too small & too light)

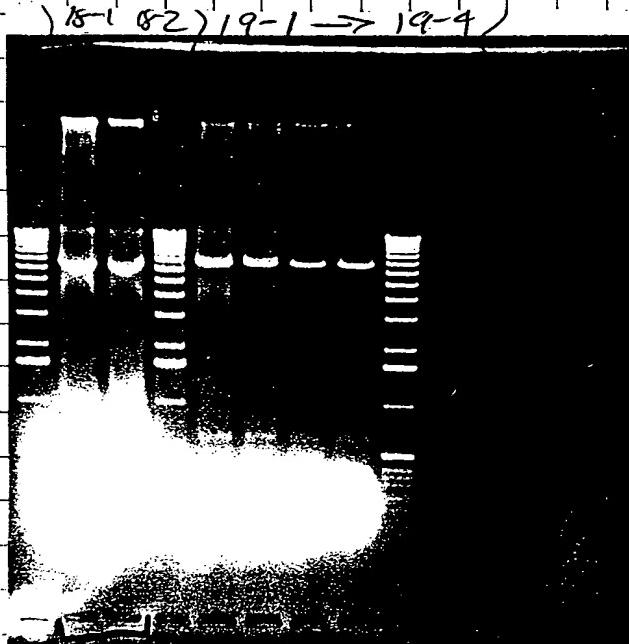
mutant

4 kb

4.7 kb

0.3 kb

DNA from date 3/10/95



H₂O = 6.0 μl.

RReact6 buffer = 2.0 μl.

mp18(1) DNA = 10.0 μl

Sst I (Sph I) 1.0 μl ea

TN 2.0 μl

H₂O = 6.0 μl.

buffer = 2.0 μl

mp18(2) DNA = 10.0 μl

Sst I (Sph I) 1.0 μl ea

TV 2.0 μl

arp 4/12/95

T Pag

Witnessed & Understood by me,

S. B. Salas

Dat

4/12/95

Inv nt d by

Recorded by

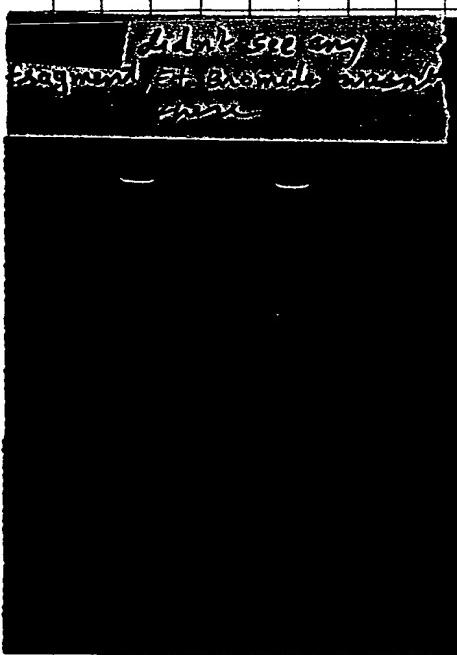
Dat

4/12/95

3/10/95 1 Sst I / Sph I 1 70.6

age No. _____

- incubated both tubes @ 37°C for 30 min.
- added 2.0 μl loading dye to each tube
- ran both on a gel
- took picture



3/1/95 T.neal mp

1.0 ml T.neal (Sph I) Imp 19 + 2899 + Sau 3AI grown for 5 hours @ 37°C in 10 different glass tubes

after 5 hours transferred 1.0 ml culture to the 10 labelled eppendorf tubes
cfg all 10 eppendorf tubes @ room temperature for 2 min.
removed supernate & saved
put all 10 tubes w/ pelled & all 10 tubes w/ supernate @ -70°C
overnight or until 3/16/95 Thursday.

TE: Brian had to leave @ 4:30 pm so this was a point to
Stop @:

To Page No. _____

Signed & Understood by me,

Date

Invented by

Date

Dolcino

4/12/95

Recorded by

Dolcino

4/12/95

132

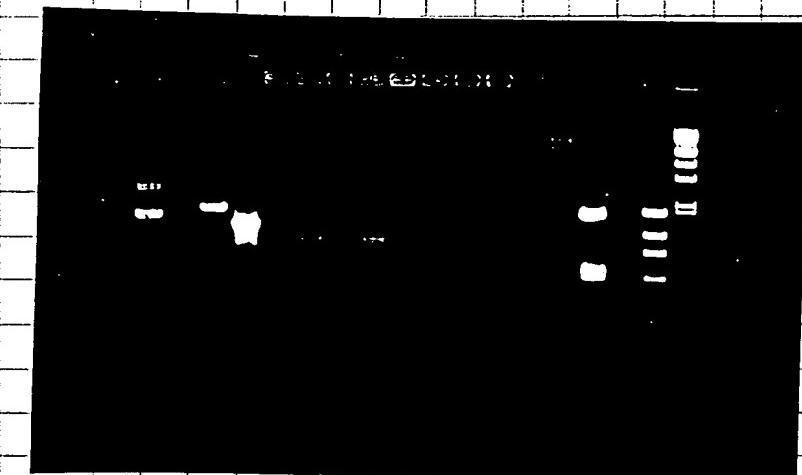
12/13/94

Project No. _____

Book No. _____

TITLE _____

From Page No. _____



1 3 5 8 13 15
2 4 9 12 14

- All samples, Vector, insects, ligation Rx run on
- Ligation seems to be worked - no signs of freezing smear.
- Lane 1 unaligned lane chnl shows up.
- Purified Vector shows some contamination.
- T + D.V 1.5 my unrun basically run.

3 purified Vector 1 ml = 100 ug still has contamination from last 2 lanes

1 1 unaligned pcr = 0.2 mg
2 2 unaligned pcr 2.5 pcr X loaded 10 pcr - nothing seen
3 3 PCR product 2 pcr 1.75 bp.
4 4 purified insect Tag, 1.5 Rx two little.
5 5 ,
6 6 ,
7 7 ,
8 8 ,
9 9 ligation 5 with purified Vector 3
10 10 ,
11 11 ,
12 12 ,
13 13 Vector absent
14 14 ,
15 15 Vector + rest before purification

To Pg

Witnessed & Understood by me,

Date 12/13/94

Invented by

Date

Recorded by

K. Bharanam

12/13/94

ge No. _____

There were 88 g blues and a few whites in 100 ml 9
 1) Tag & Tag + 2 rev. reaction plated once again the
 rest of the materials in a fresh set of plates. 1.5 mol Rx alone.

	Blue	white	2
Tag :	30.5	4	1.26 2
	9.2	1	
Tag + DV :	16.1	8	2.48 2
	20.1	1	
Color only :	64	—	

Tag + DV as more 11

picked a few blues and

6 whites from Tag

6 " from Tag + DV

Grew - nine phys.

from mix G

page.

To Page No. _____

ed & Understood by me ,

Date

Invented by

Date

12/14/84

Recorded by

K. Sitaranam

12/14/84

Project No. _____
Book No. _____

TITLE

Received 7 clones of T1/poC gene
from AR 12/12/94

94

From Page No. _____

Follow P115, 6

make 2 ml 0/10 of each in LB 100µg/ml Amp 50µg
at 30°C

0.4 40 12-13

incubate ~~2 ml~~ 0.5 ml into 2 ml culture growth
with Amp, Tet (as above)
at 30°C shaking

A555

started at 8 AM

11:30 AM 0.296
12:35 0.43
12:30 0.56

induce at 42°C 2 hr shaking at 12:45
final OD = 0.87 at 3 pm (some cells pellet)

(minipups) 7 ml from 0/10

1 1084
2 152
3 106
4 202
5 151
6 1074
7 109

see P 157-

will try induction in

42°C, 15' and

37°C 40 min

dilute in MAFI III Agar II
and Ecoli in NEB buffer 4
(see PG3) 2 h, 37°C

To Page

Witnessed & Understood by me,

Date

Invented by

Date

12/12/94

Domenica Polano 11/clar

Recorded by

Project No. _____

134

12/13/94

Book No. _____

TITLE _____

From Page No. _____

Purpose: To make more ligating reactions at a different ratio of Vector : insert + transform.

Vector	0.5	}
insert	6.0	}
5x buffer	4.0	
T4 ligase	1.0	
H ₂ O	8.5	

20 μl at 25°, 3 hrs.

Done the same for all recs

- Vector alone

- Tag

- Tag + Dependent } 1.5 ;

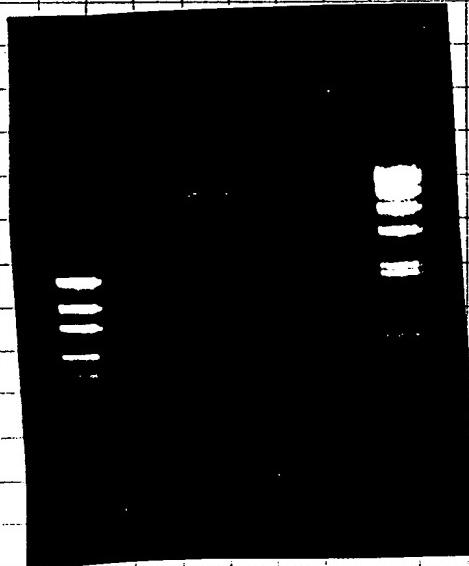
} 1

- used 2.5 μl of each reagent to transform D315 & n efficiency cells.

- included unheated pvc (negative) Control.

- usual procedure.

Ligation Recs



1 2 3 4
Vector / / /
Tag / / /
Tag + DV / / /

Vector + rest

Vector only	: recs	μl	-	51
Tag			25	μl - 160
			50	- 852
			100	- 504
				1006
				1006
Tag + DV			25	μl - 124
			50	- 274
			100	- 487
				487
				885

In this set T+DV: seems to be still a very low count.

To Pag N

Witnessed & Und st rd by m ,

Date

Inv nt d by

Date

12/14/94

Rec rd by

K. Bharaman

12/14/94

34

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

3/16/95 Thurs.

con'd from page 33 3/15/95 wed. MINIPREP

- took the pellet out from -70°C (10 eppendorf tubes)
- added 100 μl S1 mixed well
- added 200 μl S2 put all 10 tubes on ice. mixed
- added 150 μl 7.5 M NH₄OAc
- incubated on ice for 5 min.
- centrifuged all 10 tubes for 5 min. @ room temp. (4°C)
- transferred 400 μl of supernate to the new 10 labelled tubes
- added 800 μl EtOH Mixed well
- incubated all 10 tubes for 30 min. @ -70°C.
- centrifuged & discard for 2 min. @ room temp.
- discarded supernate & washed pellet with 70% EtOH.
- added 50 μl TE to all 10 tubes w/ pellet

tubes

$$\begin{array}{rcl} H_2O & - 1.0 \mu l & \times 10 = 10.0 \mu l \\ \text{buffer} & - 2.0 \mu l & \times 10 = 20.0 \mu l \\ \text{Eco 47III} & - 1.0 \mu l & \times 10 = 10.0 \mu l \\ \text{TV} & & = 100.0 \mu l \end{array}$$

- added 10.0 μl from TV to all other 9 tubes
→ added 10.0 μl DNA to each 10 tubes

- incubated @ 37°C for 30 min.
- added 2 μl loading dye
- ran all 10 samples on a gel for 1 hour @ 190 V
- took a picture

Picture on pg 35

To Pag N

Witness d & Understood by me,

Dat

Invented by

Dat

S. Polanyi

4/12/95

Recorded by

4/12/95

ag No. _____



Parent & mutant should look like
[ECO 47 III])

	Parent	mutant
Eco	8.9 kb	4.5 kb
	0.3 kb	1.4 kb
		0.3 kb.

3/16/95

1/12/95

may probably be
too light to see

NOTE: In this we could see parent & some mutant. mutant
is seen on # 5, 6, 7, 8

Pb

To Page No. _____

s d & Understood by me,

Date

Invented by

Date

Dorcas

4/12/95

Recorded by

4/12/95

Doris

Extract TfI' cells and heat
treat as per p. 115, 6

Project No.

Book No.

Results on Pg 7

Exhibit 21

Appl. No. 09/558,421

15

g N - with 1ml Tag ext buffer (P167,3) (+ PMSF and protease inhibitor)

Polyacrylamide

x TfI' fraction

(epinephrine -

mm Tris pH 9

Ammonium

Mix A

45 μ l

A or

($\frac{1}{2} \times V = \text{vol Rxn}$)
(for 27 runs)

Red iod

3-23-91
cocktail

EDTA 25 mM

2M Tris 10 mM each

72

V

54

MgCl₂

(50 mM) Cf=2 mM

18

V

27

200 μ m MDP

597

V

880

105

V

4

122

V

183

Vp = 855

(use 95 μ l/100 μ l Rxn)

Vp = 1215

use 45 μ l/50 μ l
Rxn

1 2 3 4 5 6 7 8

Stab -

II

202

5

5

5

5

5

0.5

4x5

ol 1/4

100 μ l

72 °C remove 2 μ l to 5 μ l 0.2 M EDTA at 11, 30, 60 min

Result: all clones have thermostable activity. Eg #10D is ~0.14 μ l/ μ l
Tag is ~25 μ g/ μ l (P36) and 40 mg/ μ l in Fr I (P 37)

afford on Fr I's

To Page No.

I understand by me,

Date

Invented by

Date

evening Books

1/6/95

Recorded by

12-14-94

age No. _____

minipaged 2 blues
 Tag 6 white
 DV 6 "

12/14/94

Plated again the left over from 12/13/94 transformation Re

A	CW
35	-
1 528	6
437	4
567	7
1532	17
569	4
502	3
579	5
1650	12

1.1 %

average
of
2 days

1.42

" 0.7 %

all: Once again 4 of whites are bad here. Both
 by & Tag + DV give us 1 - 2 % mutants. + these
 are not satisfactory & make a call. → Tag : T + DV = 2 : 1!
 To Page No. _____

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Dat

12/14/94

Inv nt d by

Rec rded by

A. S. Lazarus

Dat

12/15/94

Results on TFI polarity
from PGJ

Project No. _____
Book No. _____

Exhibit 22
Appl. No. 09/558,421

97

SAM	CPM1	<u>pmol</u>	<u>u/pel</u>
201	10297.00	473	0.037
102	22380.00	1028	
3	42363.00	1946	
4	25336.00	1164	0.09
106	44240.00	2033	
6	82378.00	3786	
107	36103.00	1659	0.129
8	58201.00	2675	
9	90720.00	4169	
10	39104.00	1797	0.14u/pel
11	57842.00	2657	
12	106183.00	4870	
13	4229.00	194	0.015
108	14	8062.00	370
15	17941.00	824	
16	20144.00	926	
151	17	37486.00	1722
18	18	65420.00	3006
19	23430.00	1277	0.083
152	20	43025.00	1977
21	21	71820.00	3301
22	22	37673.00	1731 $\Rightarrow 0.741$
TFI	23	63089.00	2899
+ 0.5u	24	99545.00	4575
	25	871.00	BK & D
	26	109915.00	68 C 10m/pmol

Broadband or I' Catch 5' 90°C			
5'p	mg/pel	u/mg	
201	204	0.44	83
106	225	0.49	185
107	251	0.55	235
108	220	0.48	291 u/m
159	230	0.51	29
151	23	0.51	265
152	250	0.44	190

Note: Set n 10,000 - 20,000 u/mg from TTO Tag (Fr I')
done, as see P 104 - 105, 67 and P 36-37 this book)

~~if = 5 mg/pel~~

~~100 unit/mg~~

get a 200 unit/mg in I'

~~so~~ $\times 100 \times$ less activity here

To Page No. _____

seen & Understood by me,

Date

Inv nted by

Date

seen & Polars

1/6/95

Recorded by

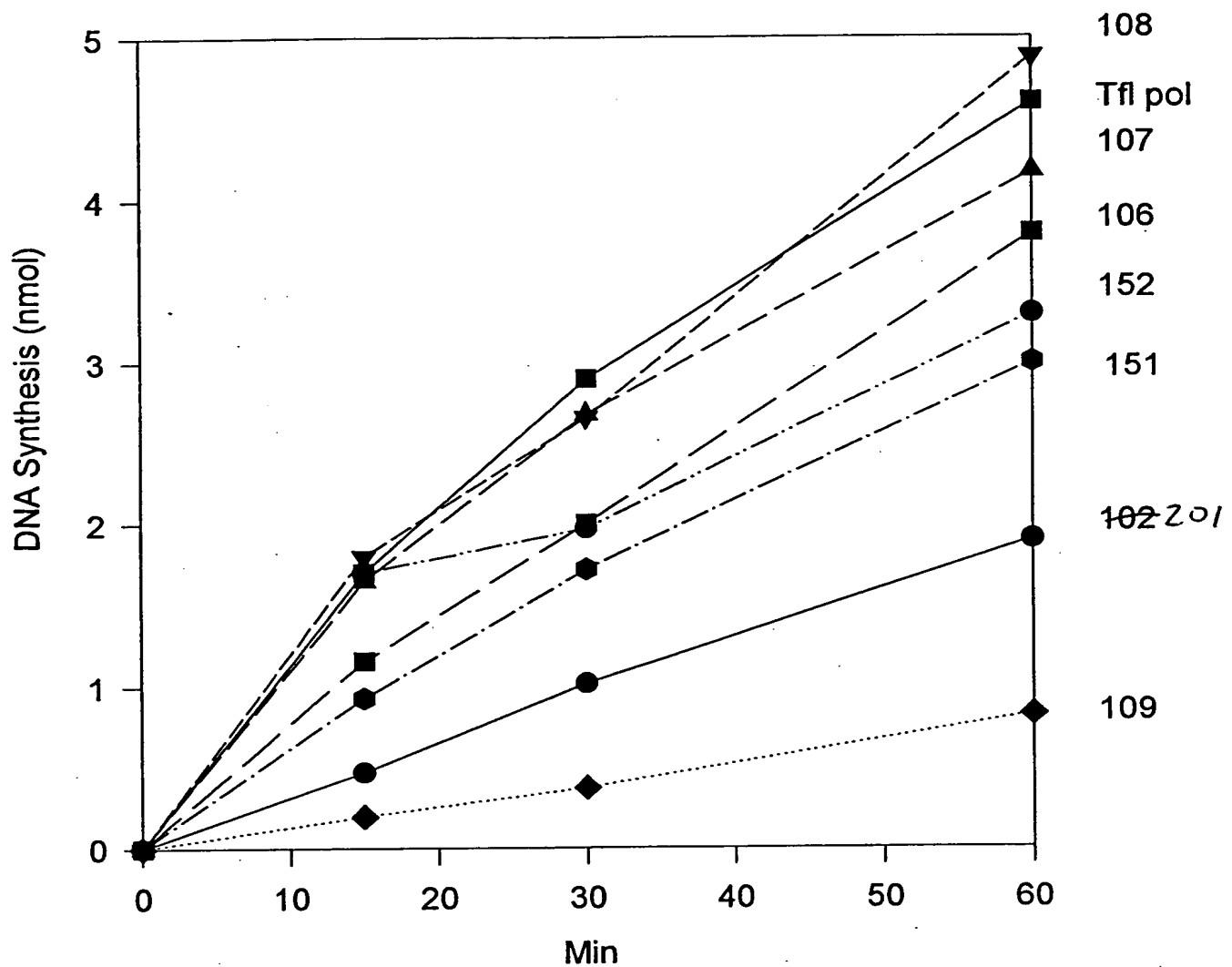
1/15/94

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

Tfl clones

Witnessed & Understood by me,

Deborah Polans

Date

1/16/95

Invented by

Recorded by

Date

12/15/84

To Page I

End label 50 μl for test
of STMP incorp opposite template U

Project No. _____
Book No. _____

99

Page No. _____ End label as per PDI P1D8, 6 (and P32, 6)

Oligo 733 (Boney)

69.4 pmol/l

↓ dil 1/69.4

= 1 pmol/l

5X kinase buffer

PNK

↓ ATP

4 μl

1 μl

✓

✓

10 pmol

2

✓

✓

8

✓

✓

21

✓

✓

40 μl

↓ 37°C, 30' → 55°C, 5'

40 μl

✓

100 pmol (50 10x excess of

678)

↓ 678 (PTray)

15 μl

✓

6.78 pmol/l

1 μl

✓

1 mM Tris pH 7.5

75 μl

✓

H₂O

75 μl

✓

132 - 60 μl

✓

(.076 pmol/l of)

32P 733

untitled.seq Length:85 Tue Nov 29 10:10:41 1994

Lower Dimers

positions: untitled:1U85 untitled:61L18

/Lower: the most stable 3'-dimer: 2 bp, -3.1 kcal/mol
AAAAAGTCACCTGCATCAGCAATAATTGTATATTGTGGAGACCCTGGAACCTATAGGAATTAAATGAAGGAGAATTCCGGTC
3' ATTACTTCCTCTM

Oligo # 678: Template

/Lower: the most stable 3'-dimer: 18 bp, -32.9 kcal/mol
AAAAAGTCACCTGCATCAGCAATAATTGTATATTGTGGAGACCCTGGAACCTATAGGAATTAAATGAAGGAGAATTCCGGTC
3' ATTACTTCCTCTTAAGGC 5'

/Lower: the most stable dimer overall: 18 bp, -32.9 kcal/mol
AAAAAGTCACCTGCATCAGCAATAATTGTATATTGTGGAGACCCTGGAACCTATAGGAATTAAATGAAGGAGAATTCCGGTC
CATATAAACCTCTTGACCTTATATCA
3' ATTACTTCCTCTTAAGGC 5'

2 μl

32P 733

To Page No. _____

Read & Understood by me,

Science Policies

Date

1/6/95

Invented by

R. Smith

Date

Recorded by

12/15/94

136

12/14/94

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

Purpose: To try Agarose in balance Rvs in transformation & whether we get better *.

L 1 = Tag 1 U (200 μg each nucleotide) 1

L 4 = Tag 4 U " " 2

Top: H 1 = " 1 U 200 μM dA + uracil DNA 3

H 4 = " 4 U " " 4

- LD 1 = Tag ± DV 1 (200) 5

LD 4 = " 4 " 6

HD 1 = " 1 (200 + 1 x 3) 7

HD 4 = " 4 " 8

- Digestion of each reactions pooled together, ethanol added after a phenol chloroform extraction

Resuspended in 15 μl reaction TE and centrifuged at 10,000 rpm overnight at 37°.

2 μl of each run on gel to see the digestion is complete.

Even though Agarose said there is enough product in PCR & some of them didn't show up on the gel after all the purification steps.

Since there is not much time to gel purify the fragments whole reaction as such, was used in the ligase reaction.

2 10 μl of it.

To Page No. _____

Witnessed & Understood by me,

Date

Invited by

Date

12/14/94

Recorded by

12/14/94

Dr. Schramm

ag N. _____

Ligation

1. Vector only
2. Vector + rest
3. Tag 2.1
4. 14
5. 11
6. 14.1
7. T + DV LD 1
8. LD 4
9. HD 1
10. HD 4

10 μl?

Restriction
dant.

Knew

exactly

how much

wasn't in there

Vector Tag Rx Tag + DV

Vector 1 μl
insert 10 μl
ligase 1 μl
76
5X buffer 1 μl

20 μl all 25°, 3 hrs.

Transformed all 10 (1 - 10 above) & Control under same con-
ditions

To Page No. _____

Signed & Understood by me,

Date

12/16/94

Invented by

Dat

Recorded by

12/16/94

S. Slaamor

Project No. _____

Book No. _____

TITLE _____

100

From Page No. _____

(1) (2) (3) (4) (5) (6) (7) (8) (9) (10)

32P T33: 678

P.G9

10X Ultima

10X PCR buffer (Taq)

1/10 20X buffer

10X Vent buffer

10X Pfu 1/1

4 dNTPs 10mM each 1 μl

Ultima 6 μl

r1 ag 3 μl EKOTK (PS09)

Taq 5 μl 11-2-94

Tfl 1 μl

rT th (PZE) 2.5 μl

Vent 2 μl

Deep Vent 2 μl

Pfu 2.5 μl

DMSO 20 μl

25mM MgCl₂

4 4 4 4 4 - - 4 4 4 4 6

H₂O

34.6 34.2 34.5 35 35 37.8 37.8 37 34.7 35

50 μl

70 °C remove 10 μl to 5 μl cycle seq stop solution
at 1, 10, 20

To Page N

Witnessed & Understood by me,

Deborah Polson

Dat

1/6/95

Invented by

Rec rd d by

Dat

12/16/94

ag N _____

~~5³²P~~ ³²P 733 Total / 50 μ l (7.6 nM primers total)

tris 10 mM Tris pH 7, 10 mM KCl, 0.02% Tween 20
 PCR buffer (BR) # Y02028 20 mM Tris pH 8, 50 mM KCl

(200 μ M dNTP)

unita pol (20.125 pmol pol molecules)

$\therefore \approx 3^{32}\text{P}733 / 1 \text{ pol molecules}$

SPFN - at 1X = 20 mM Tris pH 7.5, 10 mM KCl, 10 mM $(\text{NH}_4)_2\text{SO}_4$, 2 mM MgSO_4 , 1% Triton X-100

OTok = 20 mM Tris pH 7.5, 20 mM KCl, 10 mM $(\text{NH}_4)_2\text{SO}_4$, 1% Triton X-100

Triton X-100

To Page No. _____

Ised & Understo d by me,

enrica Polaris

Date

1/6/95

Invented by

Recorded by

Date

12/16/94

138

12/16/94

Project No.

Book No.

TITLE

From Page No. Tag:

B

W

P

Total + DR:

B

W

L 1	837	8	1	LD 1	984	2	0
L 4	777	7	1.2	LD 4	599	7	1
H 1	782	7	0.9	HD 1	732	8	1
H 4	920	4	0.4	HD 4	691	3	0

- nothing is great about!

L 1	25	156	0	LD 1	206	0
	50	210	1		341	1
	100	401	7		437	1

L 4	25			LD 4	101	2
	50	51	-		206	2
	100	119	3		290	6
	150	251	2			
	200	351	4			

H 1	25			HD 1	25	95	-
	50	92	2		50	216	2
	100	119	2		110	421	6
	150	216	2				
	200	355	1				

H 4	50	60	-	HD 4	25	113	-
	100	120	-		50	248	1
	150	315	-		100	330	2
	200	468	4				

To Pag N

Witnessed & Understood by me,

Date

Invented by

Date

12/16/94

Recorded by

R. S. Bae - man

12/16/94

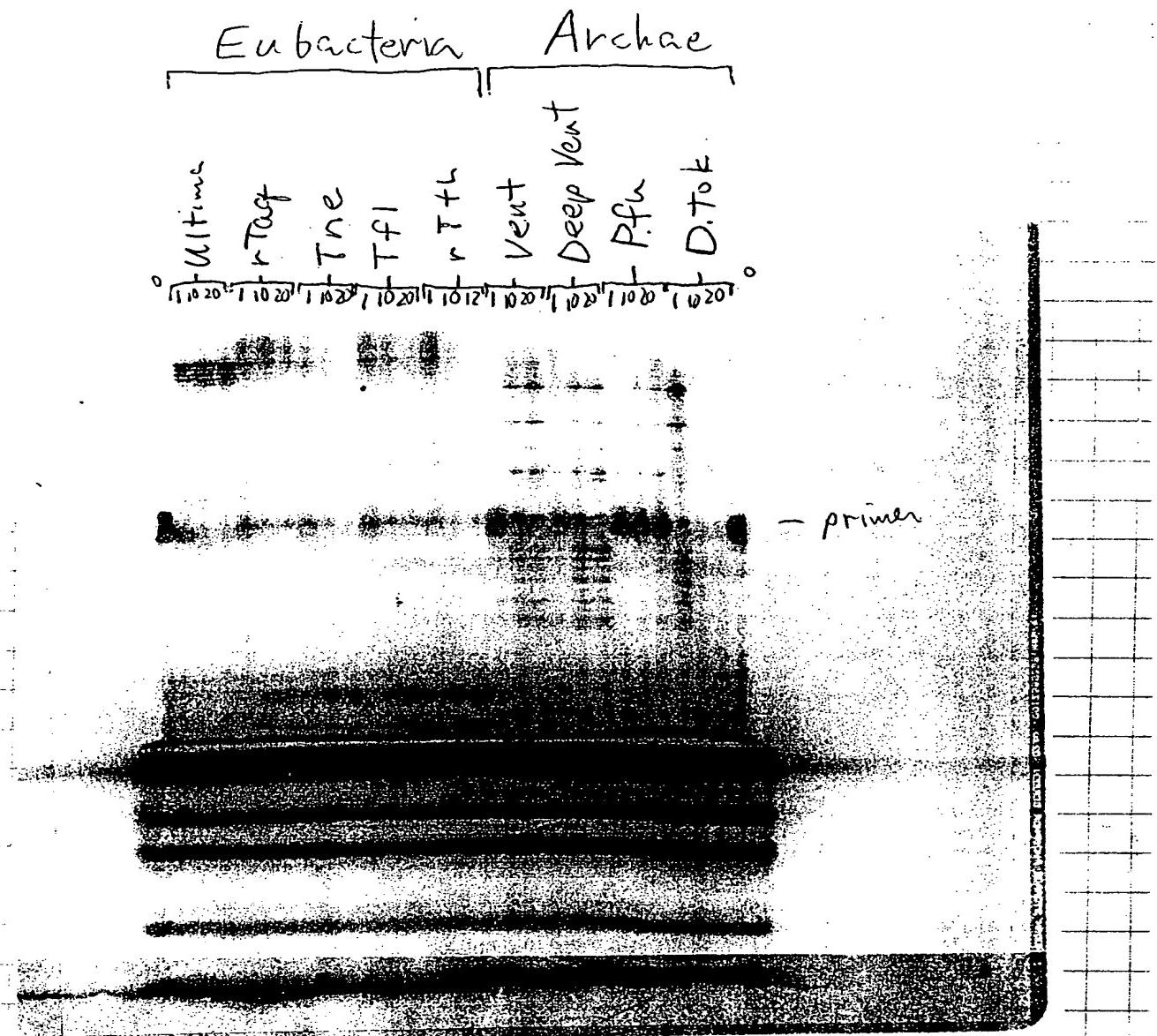
102

Project No. _____

Book No. _____

TITLE _____

From Page No. _____



999.87

0.50x Counts

- 12/17/94 - 09:42 pm

D.G.GEL

To Pag 1

Witnessed & Understood by me,

Deborah Polansky

Date

16/95

Invented by

DP

Recorded by

Date

12/17/94

date: 1/8/95 where only (-) Mn

Project No. _____
Book No. _____

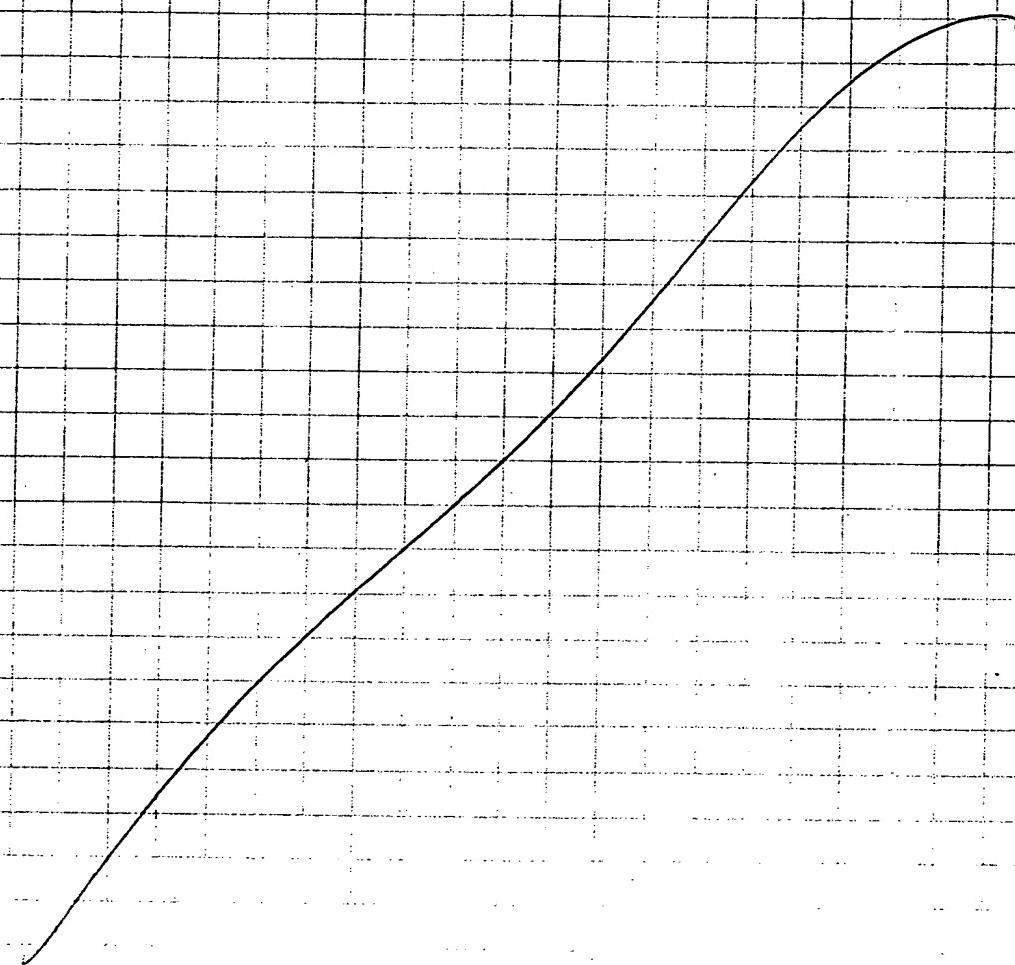
103

ag N condition gave colonies

This is to reconfirm result of pg 3 that dextro-
rotatory Mn on pVC - 19 gives full strength loss in white colonies

plates are all 0/5 = no Mn⁺⁺ and 5% Tog
per 5 into 2 ml LB + 100 µg/ml LB
30°C 3/P

Didn't complete this experiment



To Pag No. _____

d & Understood by me,

Walter Polley

Dat

2/16/95

Invent d by

Recorded by

Dat

1-10-95

Pick 3 wells from Agarose experiments
done 1/8/95 where only (-) Mn

Project N _____

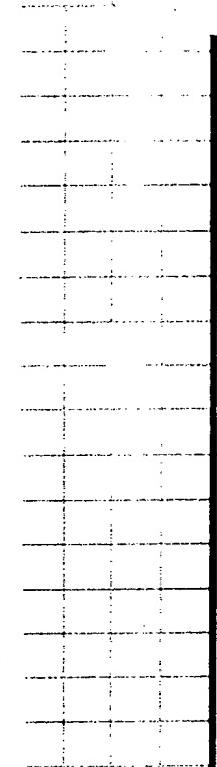
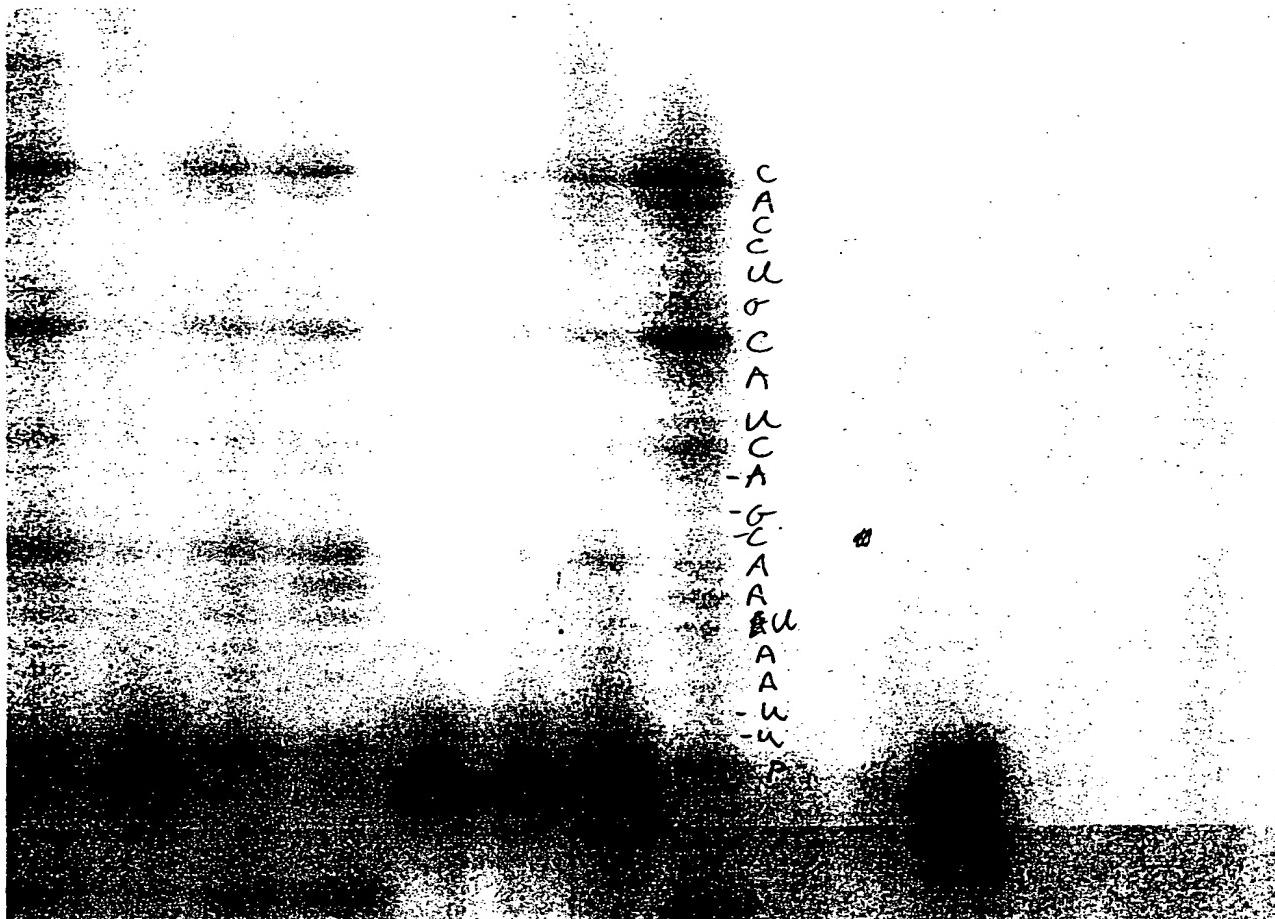
Book No. _____

103

ge N - condition gave colonies

the colonies

Taq



2.00 699.88

2.00x Counts

- 12/17/94 - 10:49 pm

d & Underst od by m ,

ananta Polap

Date

2/16/95

Invented by

Recorded by

Date

1-10-95

To Page No. _____

ag No. _____

purpose: To miniprep the bacterial cultures. To check whether all which are full lengths all solutions from alic.

1.5 ml ♂

pellet resuspended in 0.1 ml of Resuspension buffer

25-mg Tiss, 8.0

50" CDTA

1% glucose

autoclaved + stored at 4°

added 0.2 ml g, NaOH / 325 = 0.2 M NaOH

on ice 15

19° S DS

prepared fresh

added 0.15 ml g 7.5 M Ammonium acetate. Mix + incubate 15 on ice
9.15 L filtered + not pH'd.

supt + 0.9 ml g 95% ethanol. Mixed well. 5 15'

result washed with 70% ethanol 12ml. 0/0 mixing. 5 2'

expn. Vac dried 5

resuspended in 25 ml. (~ 100 mg) g TE

used 8 ml from each prep for digestion.

Reaction buffer 10x TEB 4, Aaf II, Adf II + Ecol I

90 ml 5 3 3 ml

rest 1120, volume to 192 ml

added 12 ml / Rx. digested at 37°, 3 hrs, stored at 4°.

To Pag No. _____

Issued & Understood by me,

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12/16/94

Invented by

Recorded by

Dr. S. Subramanian

Dat

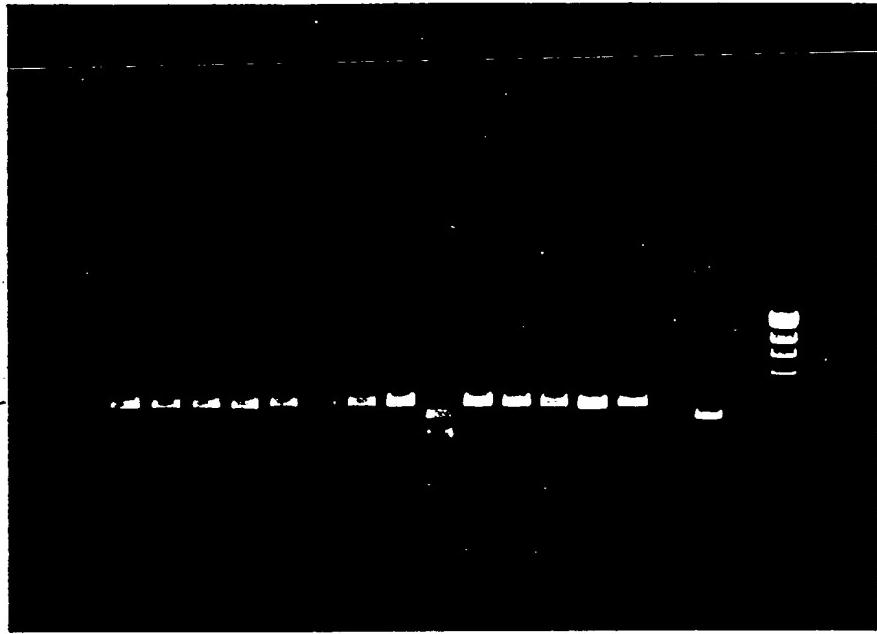
12/16/94

Project No. _____

Book No. _____

TITLE _____

From Page No. _____



8



vector

B w. from Ray w. from T + DV

Evan Palms didn't get restricted why?

Witnessed & Understood by me,

Date

1/9/94

Invent'd by

Record'd by

for disturbance

Dat

12/22/94

T Pag N

PMC9 Revisited:

Ag N.

Purpose: To repeat the experiment again with PCR for fidelity

Just :: to titrate the amount of Thermus / I used 100 - 200 pg / 3.5 cycles / 3 steps 3' extension - lot of mispairing was obvious. Today has used earlier less Thermus / 30 cycles / 2 step less of other products

so tried 0, 10, 25, 50, 100 & 200 pg of starting Thermus / 5' extension / 2 steps

With 1 U of enzyme product yield is very little - but with 2 U of Enzyme product yield was obtained

so tried with both 1U & 2U of Tag alone

To start with, in this expt Tag, Dv wasn't included

Conditions:

10x buffer K-T.

94°, 30 "

200 pg dNTP

30 (94°, 30", 60", 5')

.4 pmol primers (non-de)

2 mM Mg

Thermus 0 - 200 pg

- prepared a mix

enzyme 1 x 2 U.

12 x 45 μl

2 tubes: 1 - 12 1U

13 - 24 2U

- added 5 μl of different amounts of Thermus

To Page No.

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Date

1/9/95

Invented by

Date

Recorded by

K. S. Balaraman

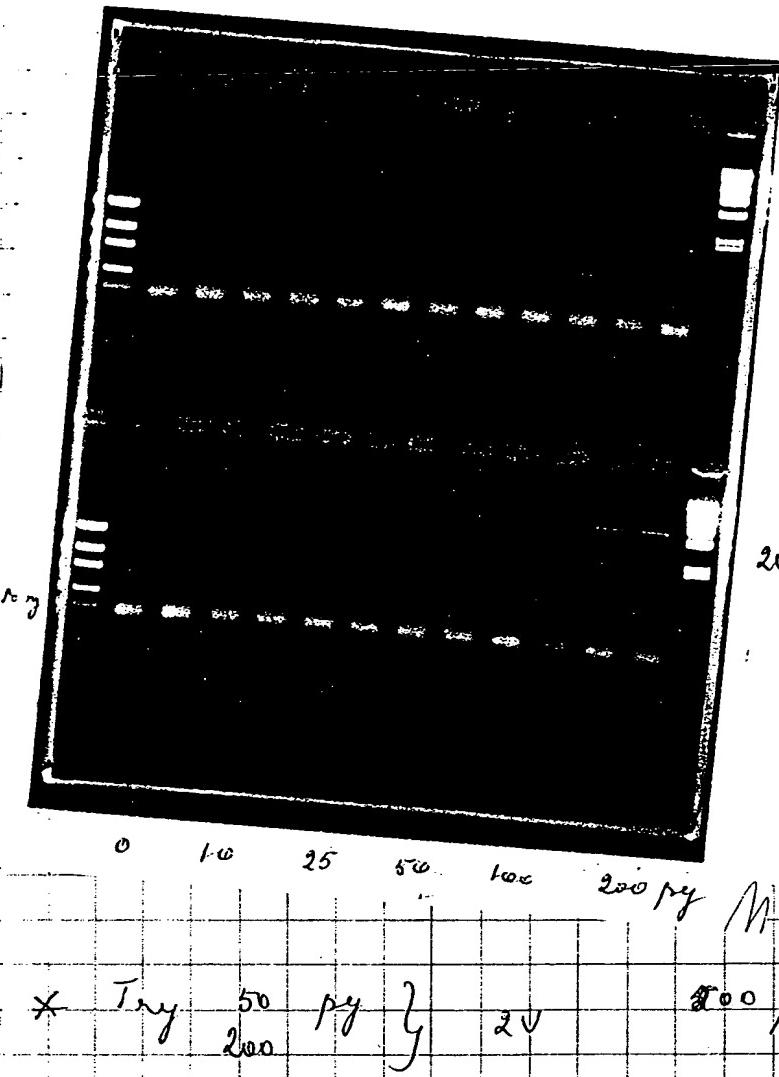
12/19/94

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

TryResult:

200 pg / 10 enzyme ✓

2.5 pg up / 2x product obtained but rather very yield even with 200 pg of starting template.

≈ 5 - 10 ng / spcl

= 31.25 - 62.5 ng /

from 200 pg tem

≈ 300 fold ampl

≈ 8 doublings!

To Page N

Witnessed & Understood by me,

Date

1/9/95

Invented by

Date

Recorded by

1/22/95

A. Sita Ramam.

Project No. _____

Book No. _____

TITLE

SDS of r Tag & Native Tag pol

From Page No. — repeat of 10-3-94 with high amounts of Native Tag

TCA appt. of Native Tag (see P96,7)

Native Tag 5 μ / μ l
lot # EAD 404

total units

H2O

15% TCA (ice cold)

	①	②	③	④	⑤	⑥	⑦	⑧
	16	32			64	72		
(80)			(160)		(320)		(640)	
184			168		136			
200								
$V_f = 40\ \mu$ l								

ice 30'
microfuge 10'
resuspend in acetone (-20°C)

microfuge 10', dried over
dry 37°C

resuspend in 50 μ l 1X sample buff

r Tag EKBT1 lot #
dilute 1/4 in 1X crack
(now its 80 μ l)

1 μ l 2 4 46

1X sample buffer 50 47 46 44

Load all 50 μ l after 5', 90°C as follows

10 μ l protein ladder

① ② ③ ④ ⑤ ⑥ ⑦ ⑧

gel same as P140, 6

1.5 mm spacers

14 wells

start 10:10 AM at 28 mA (= 72 V)

(maintain ~ 50 mA constant)

immunostain same as P140, 6

stopped gel at 3:20 5 hr 10 min total time
To Page N

Witnessed & Understood by me,

Debrae Polk

Date

2/16/95

Invented by

[Signature]
Recorded by

Date

1-11-95

Project No. _____

Book No. _____

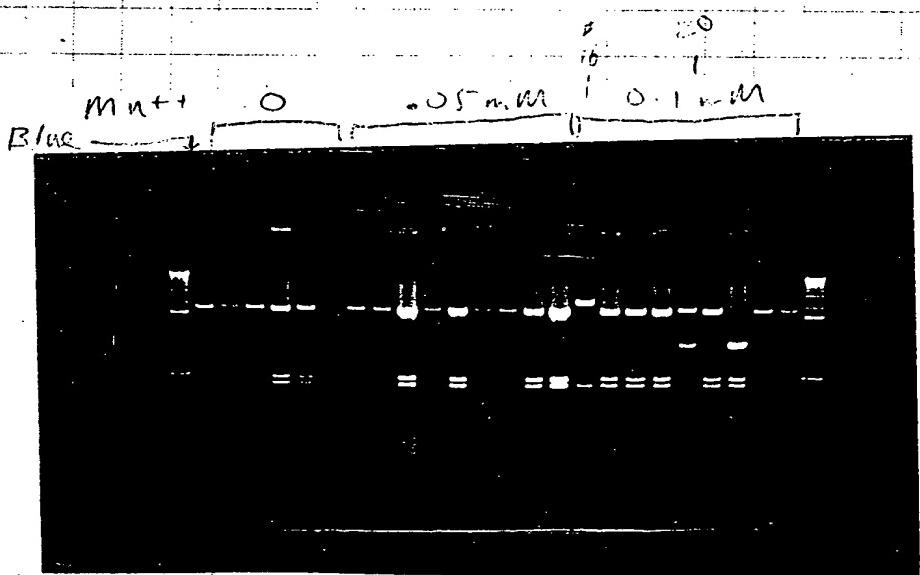
TITLE

minigels for Agrobacter plasmid
of 1-10-95 & 0, 05, 0, 1 mM Mn
for pUC PCT2
from O/N, 2nd culture, 10⁶ pg/ml

From Page No. ____

mM Mn Cl ₂ =	0	1	Blue	(1)
	0	2-6	whites	(5)
0.05		7-15	whites	(9)
0.1		16-24	whites	9

digest same as P.93, 2 hr at 37°C, AatII, AfI, EcoRI, NdeI, SmaI



AatII
465 bp →
410 bp →
AfI, R1

AatII →

MspI
183
pUC19
2676

- AatII ↑ - R1 in #2
R1 only → R1 is running
410 bp → 875 →
AfI/R1
band is present
AfI ↑ R1

Results:

for 0.1 mM MnCl₂, 3 of 9 are rearrangements;
2 are in low regions, one is in vector

0 and 0.05 mM Mn²⁺ are 9/9 full length

To Page N

Witnessed & Understood by me,

Deerana Polano

Date

2/16/95

Invented by

Recorded by

Date

1-12-94

Page No. _____

Object: Amplification of pMC9 with 2u of Tag and different amount of Dey. Vent.

prepared premix with 2u of Tag w/o any Dey. Vent.

added diff. amount of Dey. Vent., done in duplicate

Tag	D.V	µl
2u	5	2.5
	2	1
	1	0.5
	.5	0.25
	.2	2
	.1	1
.05	.5	
.02	2	
.01	1	
.005	.5	
.001	1	
0	0	1/10

$$1\mu\text{l} \rightarrow 20\mu\text{l} = 0.1\text{u/l}$$

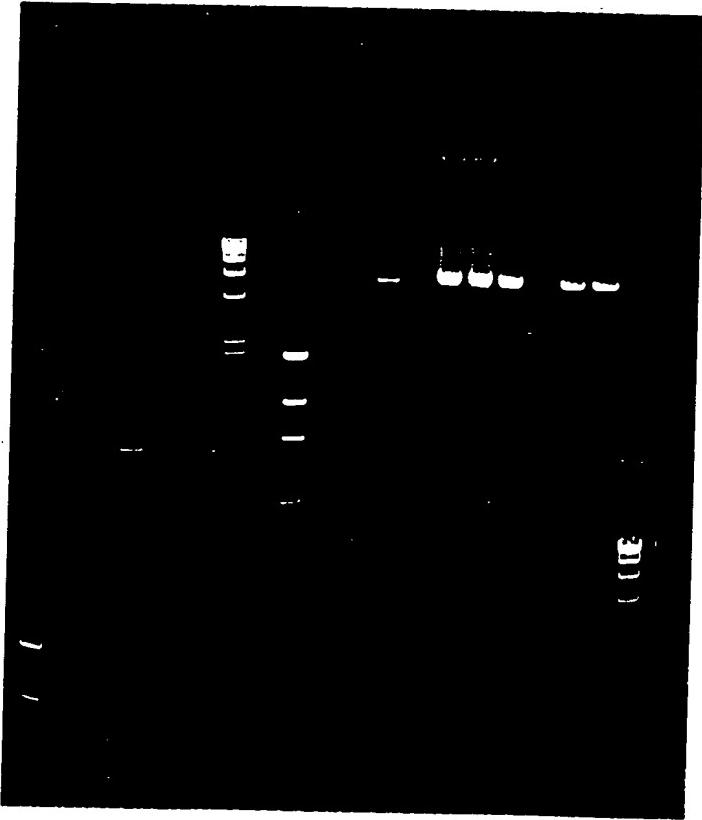
$$> 0.01\text{u} < 1/10$$

200 µg dNTP
4 µM primers
50 µg Ternphate
2 mM Mg

(94° 30')
(94° 30') 85°
(68° 5')

H ₂ O	1120
X buffer	140
Mg	28
NTP	28
reverse 1	10.5
2	11.8
Ternphate	56.0 (50 µg / Rx)
Tag	5.6
	1400

has to be expected again
new ink between
print Rx & no.



Signed & Understood by me,

Date

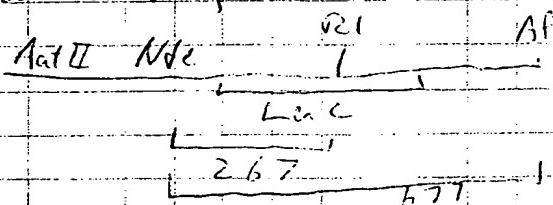
Inventor

Recorded--

A. Subramaniam

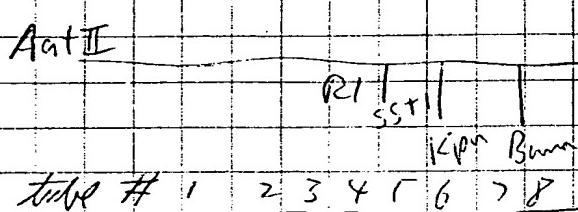
12/22/94

Age N cut on at NdeI to see if all of loc is present even though AatII is missing



NdeI is OK in buffer 4 (NEB) (P169)
expect 677 bp NdeI/ApaIII band

I cut with SstI, KpnI or BamHI to see if missing R1 site is only a point mutation (all OK in NEB buffer 4)



ApaIII

(For colony #20
(tubes 2, 4, 6 before)
see if SstI, KpnI or Bam
can cut in MCS)

tube # 1 2 3 4 5 6 7 8

A # 1 5 5 5 5

(one colony)

missing R1

missing AatII

R1

ApaIII

I

I

BamHI

S1

20

buffer 4

mult.

0.1 → -- ✓

0.3 → ✓

1 1

1 1

1 1

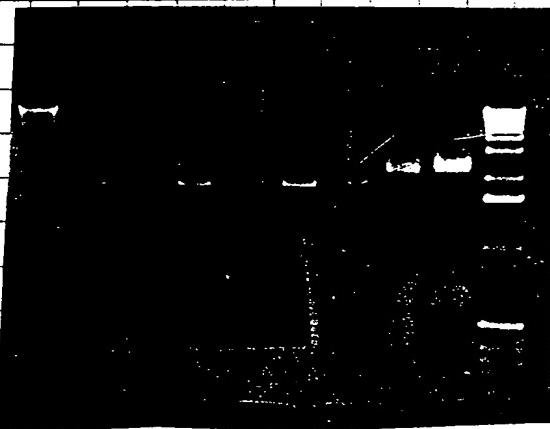
1 1

11.6 → ✓

2 → ✓

20 μl

SstI KpnI BamHI



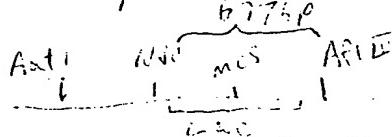
37°C 2 hr

GAATTC GAGCTC

R1 SstI

point mutation

SstI cuts but R1 did not (P106)
so mutation is no more than 1 bp downstream
from the R1 site and may even be a



To Page No.

NdeI may give the 677 bp fragment expected
if multiple bands are seen

Signed & Understood by me,
Encore PolarisDate
2/16/95Invented by
Recorded by

Date

1/13/95

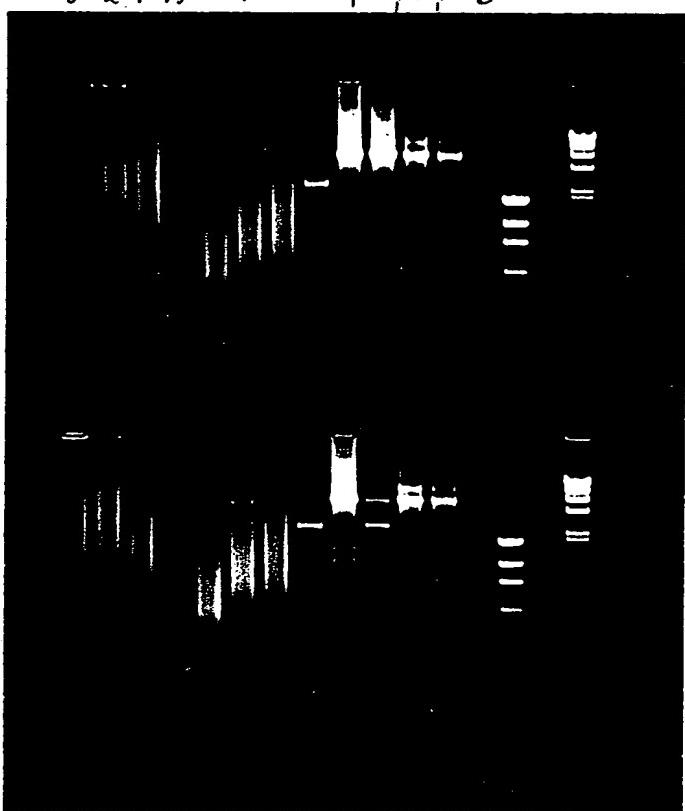
Project No. _____
Book No. _____ TITLE _____

144

12/27/94

From Page No. _____

The gel was run...



Results:

- tag 2U / 50 µg g yield
- mis running still under these conditions
- Higher conc of Dps in the presence of Tag no product
- 0.02 U + 2U Tag no product
- 0.01 U + 2U Tag plenty g product but with
- Is this Dps went too far mistake in diluting ??
- check again w. new Rx

Witnessed & Und rstood by me,

Date

12/28/94

Invented by

Record d by

J. Blahman

Date

12/28/94

To Pag

108

Project No. _____

Book No. _____

TITLE _____

Plan for IL containing ST in

From Page No. _____

2 5 10 min trial points

reactions

wl

(Want buffer in all reactions)

⑪ - ⑯ DNTP A, C, G, T for $J(A)$ vs $J(T)$, respectively, P. 8 Well

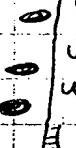
① ②

Tag 0.5u (+) H₄C₆G₂T-TP (-) JATP (dT only)

1

3

expect same before U ~



for ⑪
expect all run off

Tag

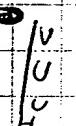
0.5

0.1 units

0.5

2.5

2.5



diff
expect
run off
for + and
off & in 5 min

③ J(T)

④

⑤

⑥

⑦

⑧ J(W)

⑨

⑩

⑪

⑫

↓

↓

↓

↓

↓

↓

↓

↓

↓

↓

same as ③ ⑦

except J(W) instead

of J(T) in 1/2 time

expect

ghosts

↓

↓

↓

↓

↓

note on p. 100 used 2.5 units Tag but will use only 0.5 units
here so that extension is stretched out over 10 min trial course

To Page N

Witnessed & Understood by me,

Devenara Polap

Dat

2/16/95

Inv nted by

Record d by

Dat

1-16-95

340 733

Project No. _____
Book No. _____

109

Stage N Same as P 99

make 40 μ l of ^{32}P 733(1)(T) ✓ (2)(A)
20 μ l 20 μ l

2863

vial #678 (JT)
10 pmol/ μ l5.05 μ l

✓

678 (GU)

6.76 pmol/ μ l
NTris pH 7.5H₂O2
407.05 μ l ✓2

✓

5.6.5

VP = 6.6

5', 80°C → cool slow

To Page No. _____

I d & Undrst od by me,

Eduardo Polanco

Date

2/16/95

Invent d by

Recorded by

Date

1-16-95

Page No. _____

purpose: PCR amplification with 20 μg enzyme + different amounts of Deep Vent.

Repeat of previous exp., 4 of points less.

200 μM dNTP

D.V.:

0.4 μM primers

1, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01,

50 μg Template

0.005, 0.002, 0.001, 0

2 mM Mg

2 U Tag

10 μl diluted to 0.1 U/μl → $\frac{1}{10} = 0.01 \text{ U}/\mu\text{l} \rightarrow \frac{1}{100} = 0.001 \text{ U}/\mu\text{l}$
in 1x buffer w/o Mg.

prepared premix 25x, done in dry-blocker.

45 μl of " + 5 μl of different amount of enzyme.

H₂O

10x buffer 125 μl

dNTP 10 mM 25

Mg 10 mM 25

primers 1 10.6

2 9.5

Template 25.0

112.5 ← added 2.5 μl Tag → 250

removed 40 μl = w/o any enzyme

After adding Tag, mixed & aliquoted 45 μl / 16 dif. tubes

added Deep Vent diluted different conc.

To Page N _____

Signed & Understood by me,

Date

12/27/94

Invented by

Date

Recorded by

K. Sankaranarayanan

12/27/94

From Page No. _____

-8 - no enzyme

1 - 4U Tag + 0.02U DV

2 - 2U Tag 0 DV

11,0

0 + 5

3 4

5 6

7 8

9 10

11 12

13 14

15 16

0 1 }

0 2 }

0 5 }

1 2 }

2 3 }

5 2 }

1 1 }

1 + 4

2 + 3

5 + 0

1 + 4

2 + 3

0.25 + 4.75

0.5 + 4.5

17 18

19 20

21 22

0.005 }

0.002 }

0.001 }

5 + 0

2 + 3

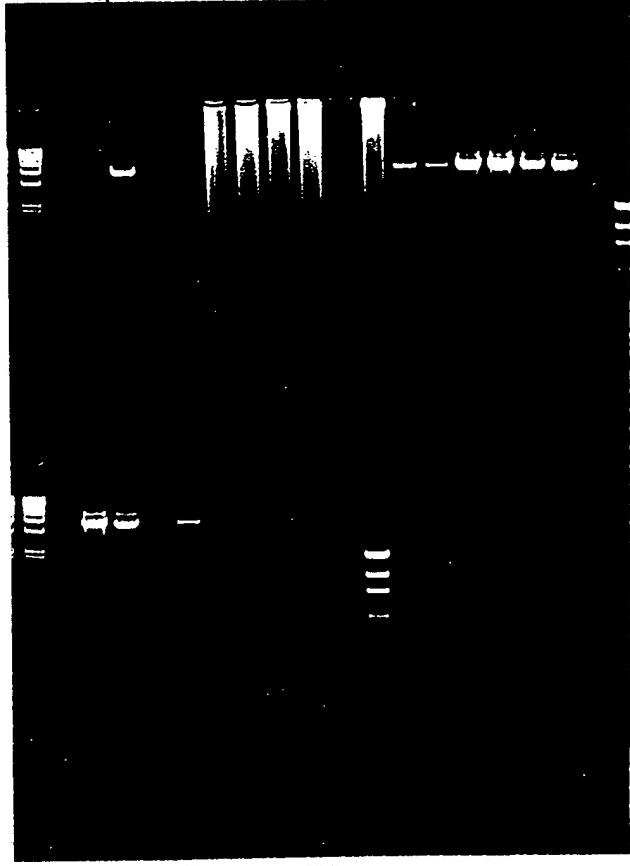
1 + 4

23 2U Tag alone = 2.

2U Tag + 0 1 5 2 1 0 5 2 0 5 1 5 1 3 1 1 9 7 5 ✓

Cycling: 94°, 30'

35 (94°, 30', 5, 6)

Result:

2U Tag + 0.02U DV

works well - In last exp. didn't work.

2U Tag alone between 1 ~ 15 in picture is very faint.

2U Tag + even .001 Ug DV
works nicely.

Optimum seems to be

2U Tag + 0.05 or .02 or .01 Ug

Degrade. But increasing the DV
to .1U gives sharper product.

2U Tagt 0.01 0.05 0.02 0.01 Ug DV.

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

12/28/94

1/11
To Page

110

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

(1) (2) (3) (4) (5) (6) (7) (8) (9) (10) (11) (12)

32P733 .2863

5 →

(dT)

32P733 - 6.78

5 →

(dN)

1.0 x Vent buffer

5 μ l →

4 JNTPA & 10 mM each

dCT, G-TP 2.5 mM each

44 μ l →

1 μ l →

rTaq 3 u/l EKBTI → 2 2 2 2 2 - 2 2 2 2 -
diluted to 0.25 units/l

* Vent DNA polymerase

0.125 u/l

1 1 1 1

0.5 u/l

1 1 1 1

2 u/l

1 1 1 1

H₂O

34 37 → 39 37 → 35

50 μ l

70 °C, remove 10 μ l to 5 μ l step at 2, 5, 10 min

pol mix

rTaq 3 u/l

2 20.67 1.65 0.5

Vent 2 u/l

* 1 2 3 *

Taq storage buffer

22 44.3 20.67 5.5

1/27 48 24 9

Add 2 1 2 3 3 1

note ix vent buffer is 2 mM MgSO₄

* dilute with vent dil storage buffer

To Page N

Witnessed & Understood by me,

Date

Invented by

Date

Dorothy Boland

2/16/95

Recorded by

1-17-95

Project N _____
Book No. _____

Tag No. _____

Purpose: To check again preq w Dependent Tag alone

50 pg template
200 pmol dNTP
.4 µM primer
2 mM Mg

0 (2+.02)
 \downarrow
 1.01

Dependent

0.51

prepared pre mix 45 µl / rx
added diluted enzymes in 5 µl.

2x DV lined: .5 1.

.2

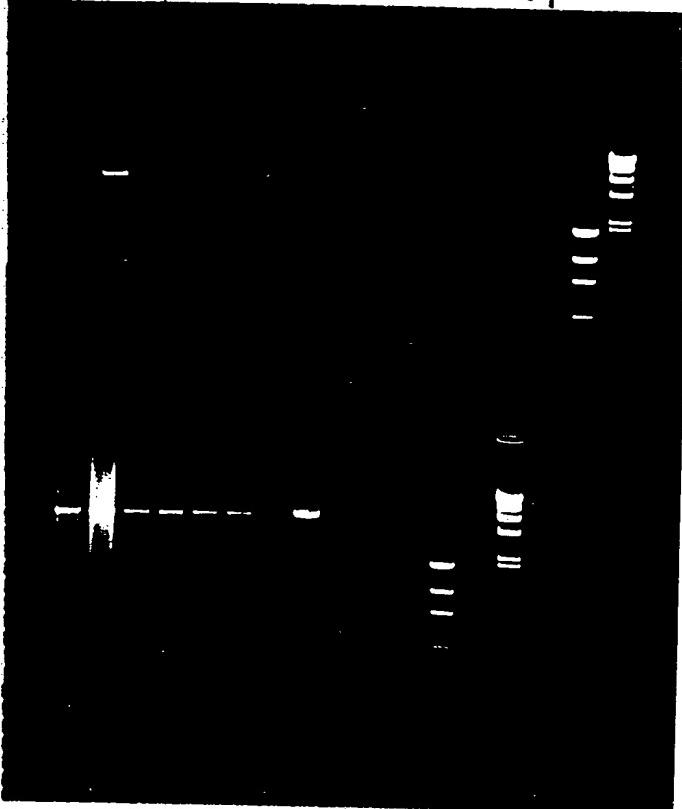
.1

.05

.01

2x Tag, 5, 2, 1.5, 0

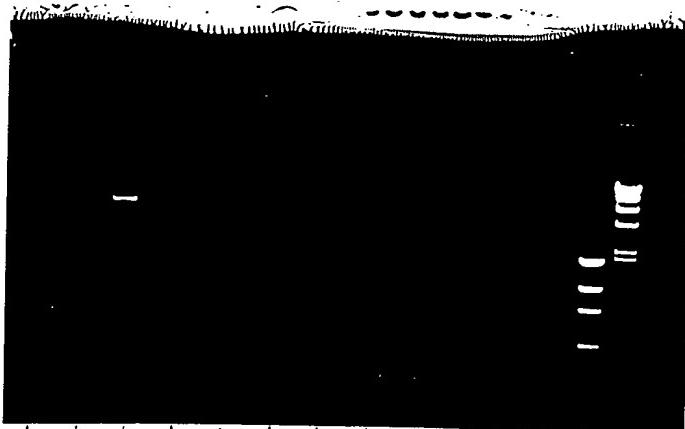
Mix: (2+.02) and (1+.01)



0.5 2 1.5 0 (1+.01)
(2+.02) mix

Increasing less than what
can I Tag one would expect
increases product
yield.

↑ order?



To Page No. _____

Ised & Und rstood by me,

Date

Invented by

Date

1/16/95

Recorded by

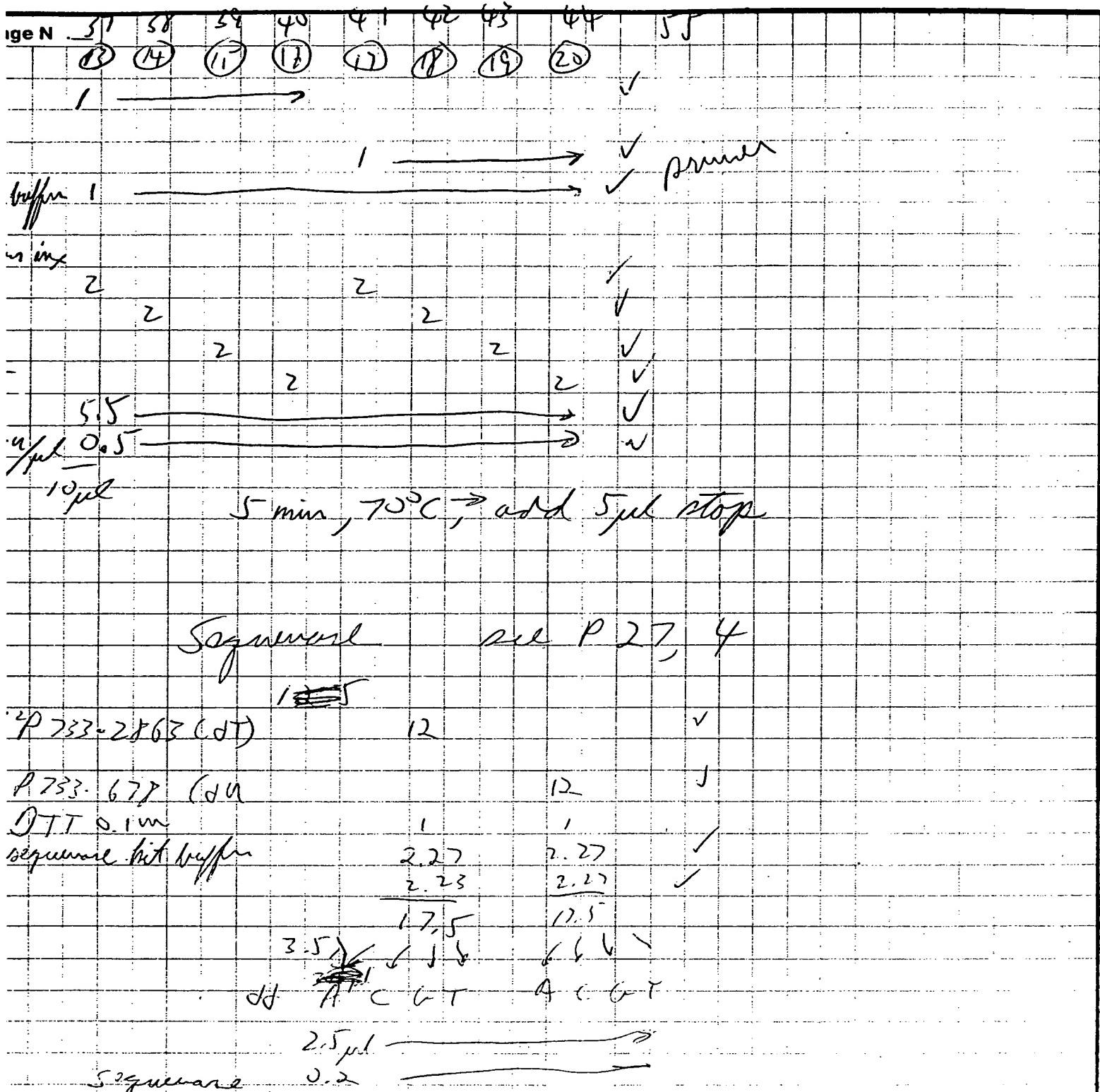
J. Stoenen

12/28/94

1d NTP sequencing reactions

Project No. _____
Book No. _____Exhibit 30
Appl. No. 09/558,421

111



To Page No. _____

Signed & Understood by me,

Suzanne Bolangs

Date

2/16/95

Invented by

Recorded by

Date - 17.55

1-18-95

148

12/27/94

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

Purpose: Mg titration in PCCP, pMC9 with Tag and Tag + DV.

add at 200 mg dNTP.

14 μM primer

50 μg template

- Mg 1, 1.5, 2, 2.5, 3 mg

(Tr-13) 2U

(2+0.02) numbered

prepared mix with Tag or Tag + DV separately 4.5

added dif. amount of Mg in 8μl

same cycling conditions.

Result:

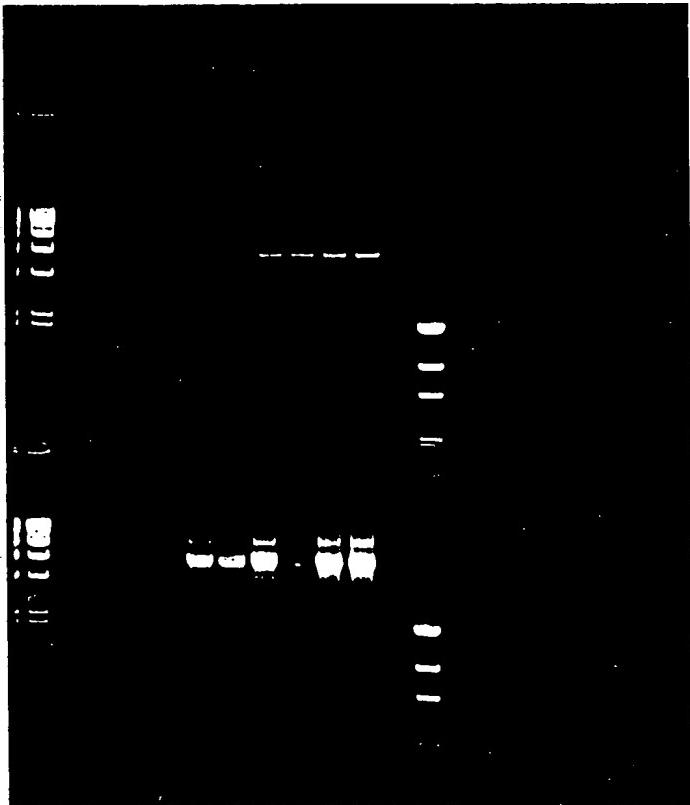
Tag 2U.

2U Tag: increase the conc of Mg to 2.5 or 3

2U considerably increase the product yield

2U Tag + 0.02 U DV even at 1.5 mg Mg product same.

more product even no more maximum with increasing conc Mg



1 1.5 2 2.5 3 mg Tag + DV
2 + 0.02

T Page

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

12/28/94

R. B. Raman

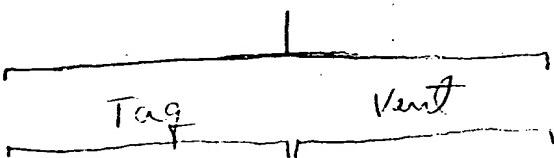
DU.GEL

- 01/19/95 - 09:20 pm

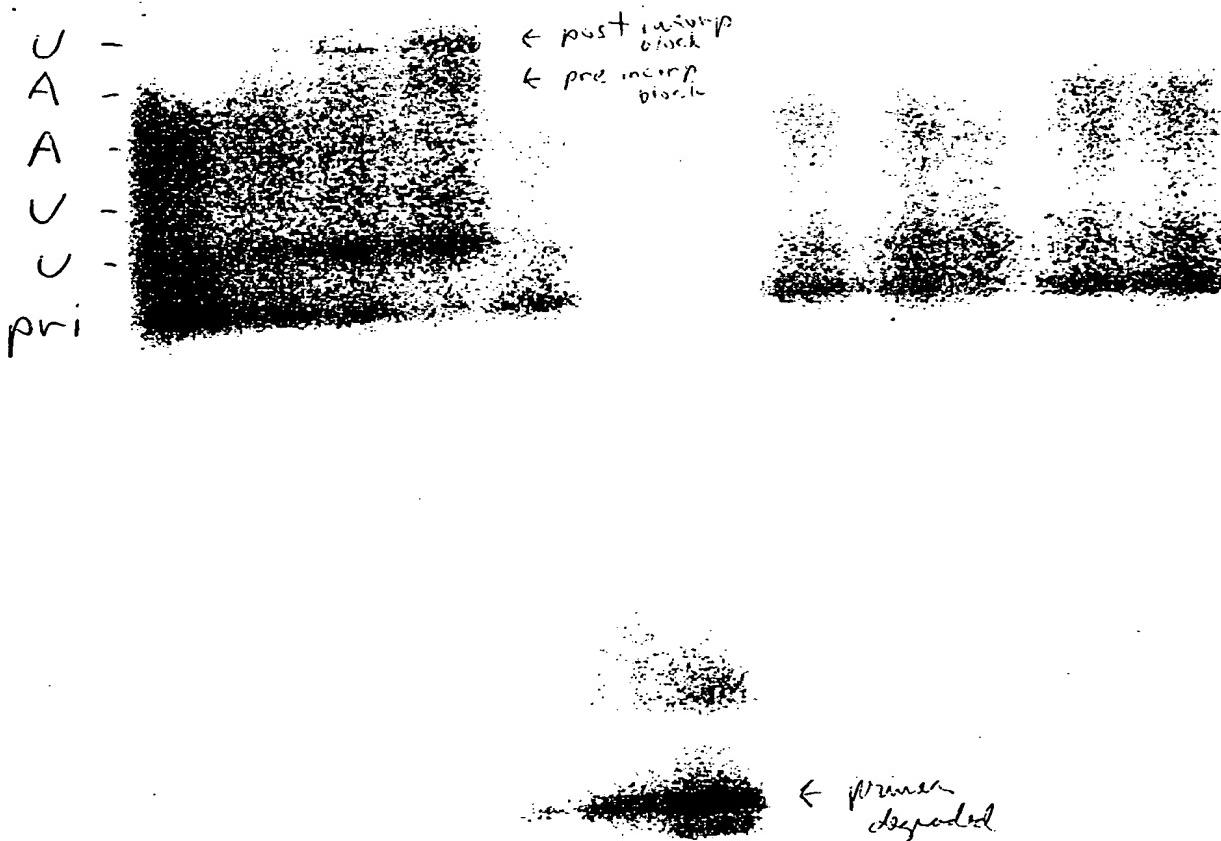
2.00x Counts

29.99 200.

- dATP and JT primer



min 0 2 5 10 0 2 5 10



Witnessed & Understood by me,	Date	Invented by	Date	T P
Recorded by				

Object No.

- purpose: To check 1 Tag dil. amount (since 1.50 works in one case)
 1. mix 1 Tag + D.V.
 2. mix 2 (Tag + DV) against
 3. freshly made (added separately)

Tube10^g Tag8^g Tube(T + DV)

2	5	4.5	14	.5	.50	+ .0050
4	2.5		15	16	1	+ .01
6	2		17	18	1.5	+ .015
8	1.5		19	20	2	+ .02
10	1	0.50	21	22	5	+ .05
11	0					

7

(added in 5μl +) 45μl rx (+ added in 5μl)

same freezing conditions.

sh10^g Tag + D.V. .005, .01, .020 - diluted in 5μl.20^g Tag + D.V. " " "

Result: w. Tag above
 10^g barely visible in procedure

- frequent freeze-thawing?



2.5 2 1.5 1 0.2 Tag

To Page No. _____

ed & Und r st od by m ,

Date

Inv nt d by

Date

Recorded by

A. Sivaraman

1/3/95

Project No. _____

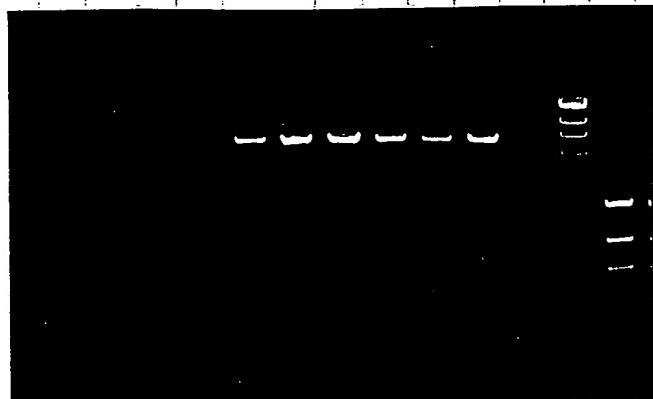
150

Book No. _____

TITLE _____

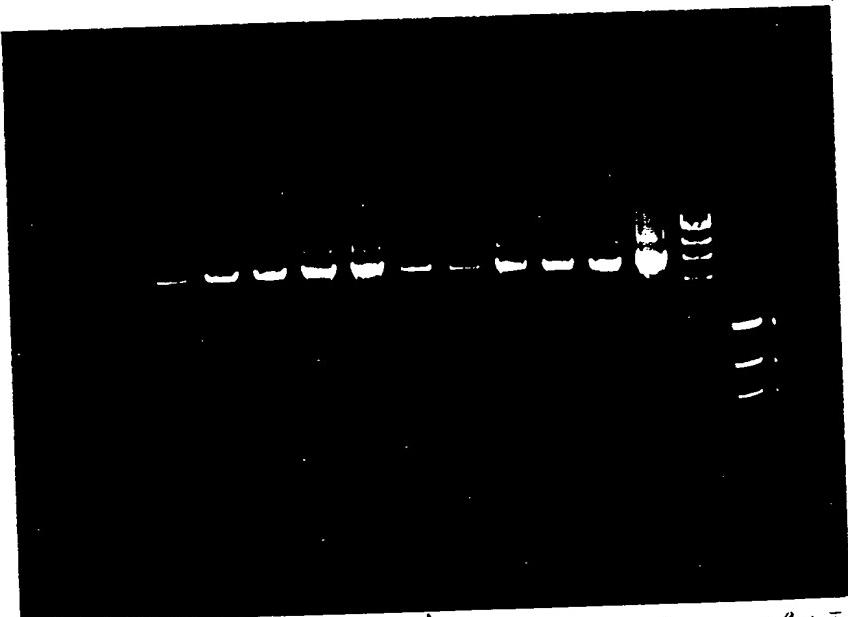
From Page No. _____

premix:



0.5 1 1.5 2 5 ml 1:10/10
0.005 .01 .015 .02 .05 10
↓ repeated again - bands ok.

fresh mix:



10 Tag + 0.005 .01 .020 1.005 .01 .02 + 2 U TiF
↓
v

Gel - Reeth with 10
unit of Tag
increasing the
amount of D
from 0.005
to 2
the band
gels up
premix seen
be holding

To Pag 1

Witnessed & Understood by m ,

Date

1/16/85

Inv nt d by

R corded by
Dr. Steinamen

Dat

1/16/85

Project No.

Book No.

TITLE

114

Miniprep for Ayoub's
PCR

From Pag No.

miniprep #

1-20

21 - 40

41 - 60

61 - 80

81 - 100

101 - 120

121

Ayoub's PCR conditions

	Taq	Taq + Dsp.I	W/N
	+		0
	+		.05
	+		0.1
		+	0
		+	.05
		+	0.1

Blue colony

grow overnight at 30°C, and add 1ml growth + 100 µg/ml Amp

miniprep small as pg1, 4 using 1 ml cells

Digest as per P 93 A IF IV Act II, Eco R1 5 µl ~~1 µl~~ miniprep
 , -40 on 4 well comb, load 10 µl
 conclude resolution not good
 enough for n 50 bp range



To Pag N

Witnessed & Understood by me,

Deborah Polcino

Date

2/16/95

Invented by

Recorded by

Date

127-55
300750A

age No. _____

1 coated (1) 1 + .01 (duplicates) }

12/28/94

(1) 2 + .02 "

(4) 2 + .1 duplicates }

50 pg

(3) 2 + .01 "

12/27/94

largest

(2) 2 + .001 "

Ray alone

2 v } duplicates

12/28/94

1 v } duplicates

12/28/94

200 pg temp

phenol / chloroform extracted 1x

ethanol optically clear night

suspended in 25 ml of 1x TG - all different amount - con
not gelled con

all 7 samples given to Judy Owen 1/4/95

To Page No. _____

seen & Understood by me,

Date

Invented by

Date

1/19/95

Recorded by

A. Schumann

1/31/95

Repeat digest of P114 for fidelity
assay: use 10 μ l miniprep

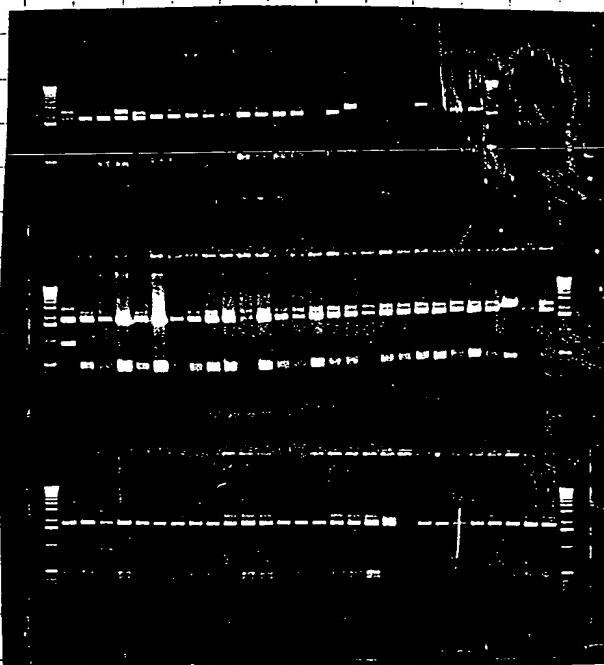
Exhibit 33
Project No. Appl. No. 09/558,421
Book No. _____

115

ag N —
NEB buffer 4 2 μ l ✓
Pfu/λ AFI III 0.3
Pfu/λ Aat II 0.1
S/λ Eco RI 0.5 ✓
Hinc II 20 7.1 ✓
 $V_p = 10 \mu$ l

Digest 10 μ l miniprep
 $V_f = 20 \mu$ l

Digest 26 37°C load 20 μ l



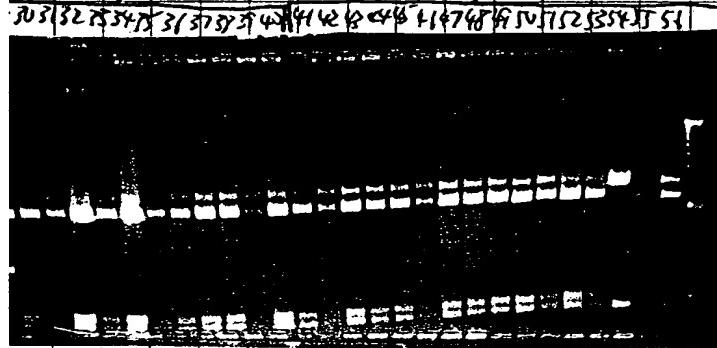
miniprep
← 57-8

← 29-56

✓
← 1-28

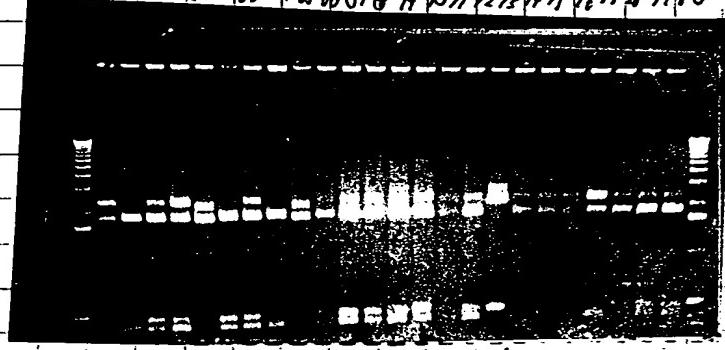
?

0.5 mM Mn 1 0.1 mM Mn



no Mn, + Vant

55159648162636486667899707132747576778711



uv

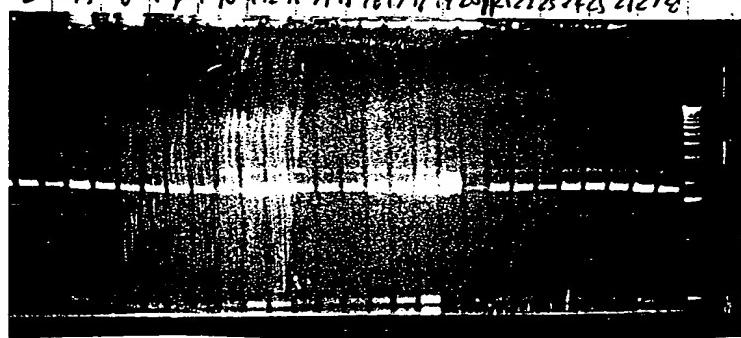
2.

31.

= 47

no Mn

0.5 mM Mn



↑ ↑ ? ?

fulling the
#13
for P123

To Page No. _____

seen & Understood by me, .

encl. Bolamp

Date

2/16/95

Invented by

Recorded by

Date

1-30-95

152

1/3/95

Project No. _____

Book No. _____

TITLE _____

From Pag No. _____

Applications:

Amplification from plasmids:

Purpose: - To start the cultures of different size plasmids in PDELTA 1.

all glycerol stocks obtained from Dilev young

6.4 kb	DYA	21	in 3 ml of LB + 100 µg/ml
8.0		57	freshly made
10.5		20	in stock of glycerol stock
20.0		47	overnight at 37°
28.0		17	

1/4/95

- except for * DYB 20 - 10.5 kb rest of them grew quite well
- . It was re-grown again overnight with fresh stock.
- Rest of them 1.5 ml of each was mini prep. using alkaline lysis method. see page 139. stored at 4° suspended in 25 µl of 1X TC.
- Each culture was diluted 10 + 990 (SOC) - 1/100 dilution plated 25 µl of each culture onto lawns made KB Amp plates. incubated at 37° overnight
- 6.4 & * 57 and * 21 were overnight but good visible colonies in the periphery of the plate
- 8.0
- 21 * 17 gave just 4 colonies in 2 plates!
- 2. * 47 gave a few with spread, full shown like colonies
- Replated further diluted * 57 & 21 for future use.

To Pag

Witness d & Understood by m ,

Dat

1/6/95

Inv nt d by

Dat

Recorded by

R. Sharman

1/5/95

Project No.

Book No.

116

Results P 115

From Page No.

#1-20, Tag 0 mM

full length TITLE + 410 bp + 465 bp (?) (*) (†)

(no AatII) (no A) III deletion 90mer NO

17 19 (see p 123 SstI)

i

21-40, Tag 0.5 mM Mn

17 20

41-60, Tag 0.1 mM Mn

18 2

61-80, Tag 0 mM Mn +Dvent

12 17 X 1 2

81-100, Tag 0.5 mM Mn +D Vent

17 2

111-120, Tag 0.1 mM Mn +D Vent

16 4

See well Table on p 124 after DntII and SstI cuts

(*) 90mer ladder R1 site in mice
(?) no result, i.e. not enough DNA to be sure about cut.

0.1 mM Mn, Tag + Deep Vent

,09 120

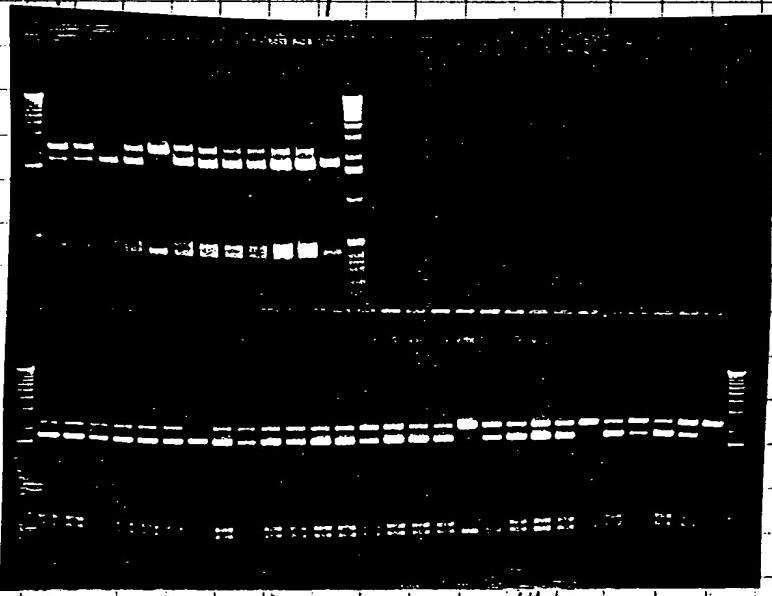
confirmed deletions

miniprep #19, b1, 6T

only 410 or 465 removed
so its 2,2 bp

1.8 bp
was 410 and 465 removed

miniprep



1 2 3 4 5 6 7 8 9 10 11 12
0.05 mM Mn Tag + Deep Vent 0.1 mM Mn Tag

Witnessed & Understood by me,

Deborah Polans

Date

8/16/95

Invented by

Recorded by

Date

8-31-95

P116 continued

Experiment done on P. 123

Project N. _____

Book N. _____

111

ag No. _____

Still Needed

cut with DraT to see if full length lacZ is present
(assuming either AF1III or AatII recognition region
had a point mutations generator). Therefore the "41" and "46" by

miniprep # 54, 58, 64, 73, 87, 98, 103, 108, 113, 125

plus AatII, AF1III

cut with SSP1 to see if R' site in MCS was
a point mutation (or very small deletion)
(all on P107 at bottom) resulting in the "90's"

miniprep # 3, 29,

Recut with 17 μ l miniprep and load 30 μ l?

$\frac{2.5 \mu\text{l reaction}}$

trying to resolve the "No results"

miniprep # 20, 39, 71, 74, 75, 76

To Page No. _____

s & Understood by me,

me and Bolano

Date

2/16/95

Invented by

Recorded by

Date

1-31-95

Project No. _____
Book No. _____TITLE Work at Frederick

From Page No. _____

SPOT	CPM's	TIME
A ¹ [1 2 3]	4976.00 3216.00 } 4797 4500.00 }	0.50 0.50 0.50
A ² [4 5 6]	16920.00 17020.00 } 16699 16156.00 }	0.50 0.50 0.50
A ³ [7 8 9]	3926.00 3822.00 } 3934 4054.00 }	0.50 0.50 0.50
A ⁴ [10 11 12]	15974.00 16520.00 } 15991 15478.00 }	0.50 0.50 0.50
A ⁵ [13 14 15]	4684.00 4752.00 } 4681 4606.00 }	0.50 0.50 0.50
A ⁶ [16 17 18]	17622.00 16806.00 } 17390 17742.00 }	0.50 0.50 0.50
L ¹ [19 20 21]	4186.00 3966.00 } 4046 3986.00 }	0.50 0.50 0.50
L ² [22 23 24]	14842.00 14704.00 } 15055 15620.00 }	0.50 0.50 0.50
L ³ [25 26 27]	4458.00 4644.00 } 4357 3970.00 }	0.50 0.50 0.50
L ⁴ [28 29 30]	16730.00 16914.00 } 16442 15684.00 }	0.50 0.50 0.50
L ⁵ [31 32 33]	4864.00 5020.00 } 4907 4538.00 }	0.50 0.50 0.50
L ⁶ [34 35 36]	15236.00 17922.00 } 17018 17898.00 }	0.50 0.50 0.50
Aquasol blank [37 38 39]	12.00 16.00 16.00	0.50 0.50 0.50

*Aquasol
blank*

delivered 10µl with p10 (wiped tip)
 rinse 3x into 4 ml aquasol

each dilution had 3 µl of 1 µCi/ml ^{3}H TTV

To Page 1

Witnessed & Understood by me,

Deborah Polanyi

Date

2/16/95

Invented by

J.P.M.
Recorded by

Date

1/25/95

New rTag dilutions

Project No. _____

Book No. _____

119

g N _____

#

EKBT1

77.4

18.6 μ l323 units/ μ l (P91)

Tag dilution buffer

4922.6 μ l1981.4 μ l $V_f = 5 \text{ ml}$
(5 units/ μ l) $V_f = 2 \text{ ml}$
(3 units/ μ l)

book are labelled "1-31-95 rTag"

To Pag No. _____

s d & Und rstood by me,

Suzanne Polley

Dat

Inv nt d by

Dat

2/16/95 R c rded by

1-31-95

6.4 kb.

Book No. _____

Page No. _____

purpose: to amplify 6.4 kb and 8.0 kb from plasmid
used F + R (non dG) primers

5.0 μ l Rx. 200 pmol dNTP each
.1 pmol primers

.2 mM Mg used buffer B (94°, 30")
Template ?
1 μ l enzyme pre mixed (1:0.01) (60°, 45")
72°, 3'

cycling: 94°, 1'

I prepared enough primers for 20 Rx:

6.4 kb

all done in duplicate.

included purified }
prep at a known } concn Cuvet 50 μ g + 100 μ g
concentration. (tag 30) just one.

mini prep, unknown concentration (from

the amount of colonies in 1/100 dilution)

Cuvet should be quite

high in the mini prep.

diluted to 50 μ l

used .5 μ l and 1 μ l

plasmid - picked a single isolated colony directly
into the reaction mix containing all the rest of the reagents
done in duplicate

8.0

no purified stuff available

mini prep - lot of colonies } .5 and 1 μ l
unknown conc } from 1/100 \rightarrow 25/ μ l } (out of 60 μ l from
dilution 1.5 ml culture)

plasmid - 2, one in each - done in duplicate

T Page No. _____

Assessed & Understood by me ,

Date

Inv nt d by

Date

1/10/95

Recorded by

1/15/95

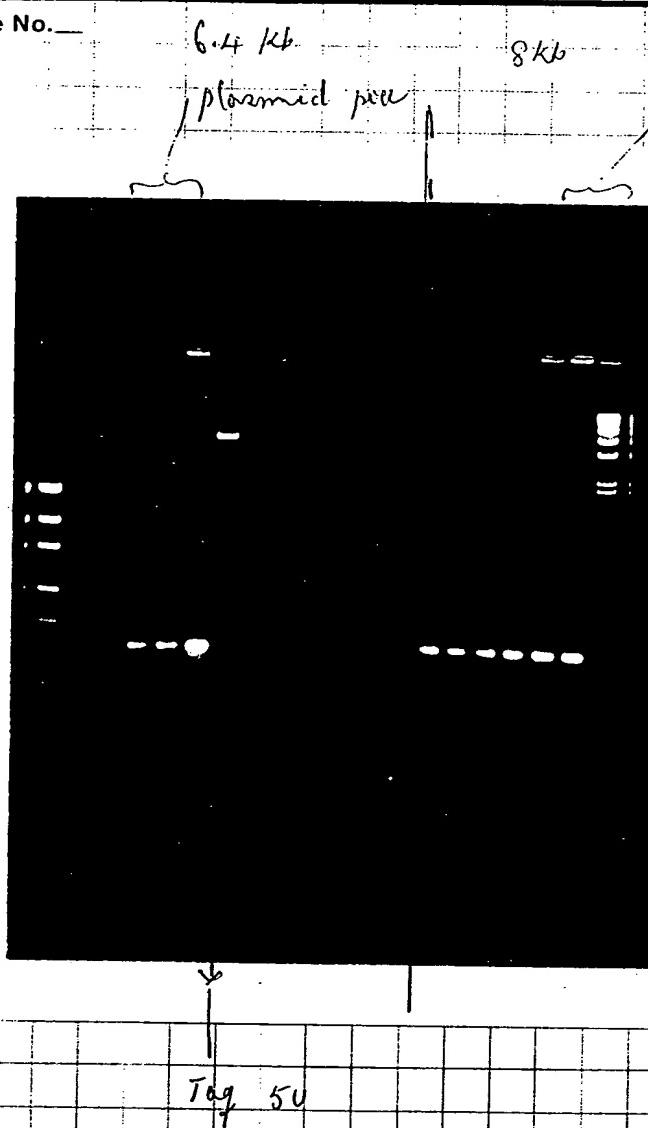
J. Sitarowski

Project No. _____

Book No. _____

TITLE _____

From Page No. _____



* check alternative cycling conditions
to get rid of mis priming.

* lysis in PK and just review has been checked next

* make 6.4 kb to work first.

Results:

- 6.4 kb gel - amplified with lot of mis priming
- Taq 5U gave good amplification.
- nothing else seen in colonies, but a stuff stuck up in the tube
- Run same tube with Sol 6 kb + 8 kb
- not a good way to do lysis at all,
- whenever there were no products lots of primers
- amount of primers, 15 μl good enough.

Witnessed & Understood by me,

Date

Invented by

Date

T Page No.

Recorded by

Dr. Bhagwanan

1/9/85

Project No. _____

Book No. _____

TITLE

Accuracy of delivering 1 μ l
with P2 pipetman for Tag storage

From Page No. _____

add 1 μ l, 10 times to a weigh boat with a drop of H₂O in it so tips can be rinsed several times. Use storage buffer at 0°C (on ice)

+H₂O Tare 0.0000

1 μ l

2

3

4

5

6

7

8

9

10

0.0119 ($\frac{94}{100}$) = 0.011

note 10 μ l SB = 0.01
0.0094

$\therefore \approx 1.1\mu$ l was added instead of the 1 μ l intended

concluded ~~2 μ l~~ 1 μ l is OK to add to unit assay

concluded 2 μ l is better to add for unit assay

stock for Tag unit assay use CF amount for 667 Rxns

3 mM 0.5M TAPS pH 9.3 150 μ l 2.5 mM \rightarrow ~~W~~ X 206

120 μ l 1 M MgCl₂ 6 μ l 1 mM \rightarrow 0.1 M OTTIV ~~X~~ 3.

1.0 μ l 3 M KCl 50 μ l 50 mM \rightarrow 1.0 mM KNTPS ~~X~~ 6.

~~1.0 mM KNTPS + 1.0 mM MgCl₂~~ ~~1.0 mM KNTPS + 1.0 mM MgCl₂~~ ~~1.0 mM KNTPS + 1.0 mM MgCl₂~~

~~1.0 mM KNTPS + 1.0 mM MgCl₂~~ ~~1.0 mM KNTPS + 1.0 mM MgCl₂~~ ~~1.0 mM KNTPS + 1.0 mM MgCl₂~~

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Witnessed & Understood by me,

Deborah Polans

Date

2/16/95

Invented by

Date

2-1-95

Recorded by

6.4 kb

Project No. _____
Book No. _____

1/6/95 155

ag No. _____

Purpose: To repeat & optimize preliminary. 6.4 kb.

Tried mini-prep Davis as a control

will try 2 dif. cycling conditions 3 step as well as 2 step.

Cultures will be lysed in 2 different ways 1. in PK (single tube)
2. in H₂O colony buffer
mini-prep will also be included again.

conditions: Since 200 μl of DNA + 2 mM Mg + 1 mM EDTA pH 7.5, 10 mM Tris-HCl worked with Tag 5W, the same conditions will be used.

need 2 μl g miniprep - can unknown (and 13410 (3)).
still have to wait after

Tried dif. enzyme conc 1:2, 5 and 1:10, 2:10, 5:10

Tag

Tag 5W

Colony lysis: Since these colonies were so small after 37° pooled 5 or 6 colonies in a single area - spotted 2 μl g lysis buffer or H₂O mixed & pipetted out the liquid onto a tube containing 13 μl g lysis buffer or H₂O.

colonies in PK lysis: 55°, 15', → 95°, 15'

in H₂O: 95°, 15'

(Added a 5 μl of H₂O) pooled all three tubes together and made up the volume to 50 μl.

Should have picked more for more reactions.

used 10 μl / Rx - approximately either PK lysed or H₂O lysed & when likely 10 μl / Rx To Pag N.

Ised & Understood by me,

Date

Invented by

Date

1/6/95

Recorded by

S. Subramanian

1/6/95

Project No. _____

156

Book N. _____

TITLE _____

From Page No. _____

For miniprep DNA : prepare premix with template

For colony : add them later

miniprep premix : 25 x

A

dNTP 25 μ l (200 μ M each / Rx)
 F P 5 (20 μ M) 0.4 μ M

R P 5T 0.4 μ M

Template 2.5 \times 2 μ l / Rx

miniprep

H2O 415

500 \rightarrow 25 μ l / Rx

Premix B: 5x

Tag

Tag + DN

(2 mM) Buffer B 1 2 5 1:10 2:02 5:08

100 μ l x enzyme

H2O

50

5 10 25

.5 1 2.5 99 97.5 95 90 75

150

20 μ l / Rx

step 2
3 cycle

2 step:

94° 3'
20 (94° 45"
55° 30")
72° 3'

94° 3'
94° 45"
68° 5'

20

T Pag 1

With ssed & Und rsood by m ,

Date

Invent d by

Dat

1/9/95

Record d by

Dr. Maranan

1/6/94

~~is & Understood by me,~~

Date /

Inv nt d by

Dat

[Signature]

19/95

10 of 10

1/6/94

Recorded by
K. Sitaraman

158 (4 p.)

Project No. _____

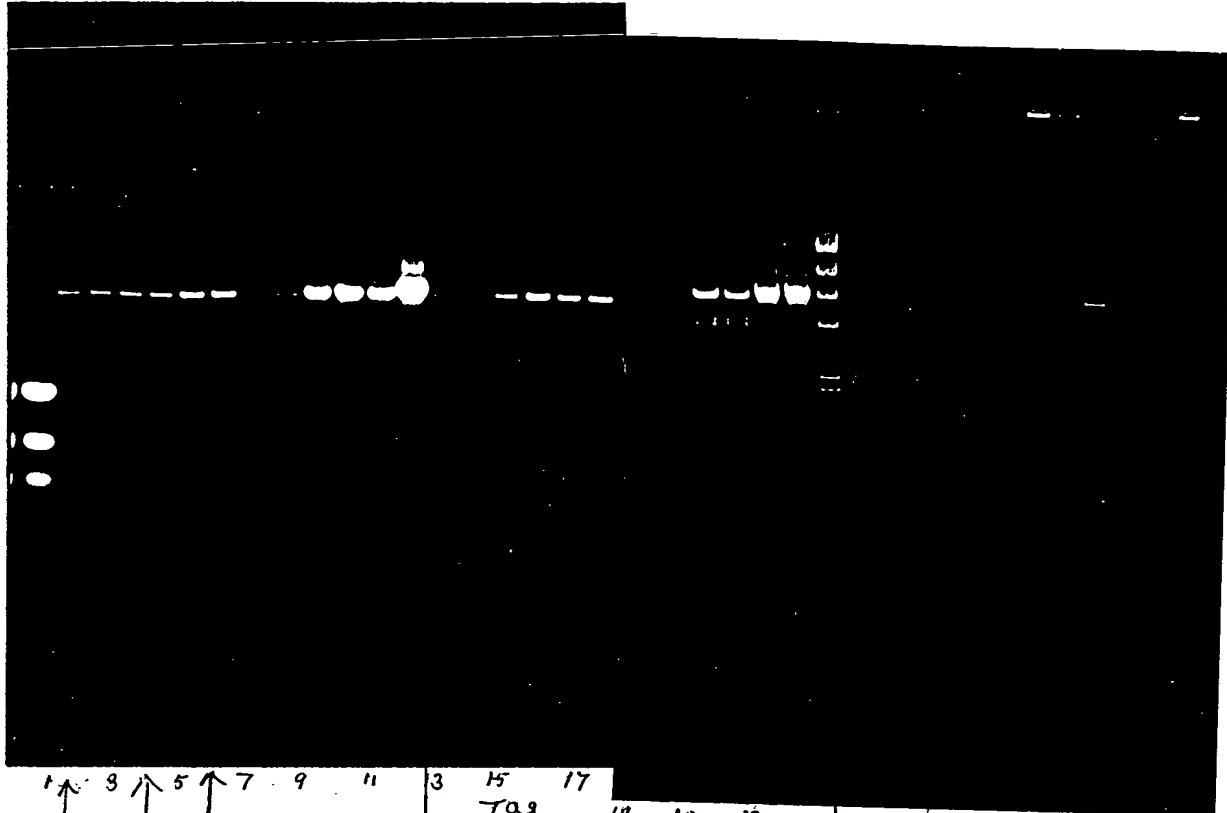
Book No. _____

TITLE _____

From Page No. _____

Step
2 Cycle

3 step cycle



1 ↑ 3 ↑ 5 ↑ 7 ↑ 9 ↑ 11 ↑ 3 ↑ 15 ↑ 17 ↑
tag

19 29 23
T P D V

Unit 11

5 1.25

empire

Tay 2663

mini prep.

plasmids

Result: Even 3 step gave better product with less mis-
plasmid amp should be done under more control.

To Page 1

~~Witnessed & Understood by me,~~

Date

Inv nt d by

Date

Recorded by

K. Staerman

1/9/94

⁸ Continued from P 111

Exhibit 36

Appl. No. 09/558,421

Project N .

Book No.

123

STI and DSI cuts of mutants

age N	run#	control	control
1	1 2 3 4 5 6 7 8 9 10 11	1	
upt#	30 54 58 64 73 87 92 113 118 120 30	12 13 14 15 16 17 18 20 21	22
buffer 4	10 10 <u>ul</u>	10 → 25.6	>
I	2 2	2 → 3	v
	1 1		
T III		✓ 0.3 → 0.45	v
T II		✓ 0.1 → 0.15	v
T		✓ 0.5 →	
-R I		✓ 0.75 →	v
S	7		
	7	✓ 7.1 →	—
Vp = 20 <u>ul</u>		Vp = 20 <u>ul</u>	30 <u>ul</u>
37°C 2 hr			
was only ~ 1/2 out by 1 hr.		control	control
add 0.5 ml more water	And T = 20.14 (20)		control

was only $n \frac{1}{2}$ cut by 1 hr.

add 0.5 ml more *easy*

for Ord I

miniproj # 6 p
Programs

control 2.7 kb (uncut), 1.8, 0.8 full length
#73 " " " csp present "

458.34
87,120

54, 18105
113

18

minigress
3 and 2/3
have full
length loc
based on previous
of 41, + 65.6%
30 ft was probably
small or point
nudation

all no
results
of P.I.
all full
length
loc based
on pictures
of 410 465 bp
when more DNA
cut here

↑
mine
#15
has 100 by
so
fall back
low in
present

To Page No.

ed & Understood by m

enrich Polans

Date

2/16/95

Inv. # 436

Recorded by

Data

Date
2-24-15

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

full length percent
lac rearrangements

miniprep #	Mn (mM)	Deep Vent	full length	percent rearrangements
1-20	0		19	5%
21-40	.05		20	0
41-60	0.1		18	10%
61-80	0	+	17	15%
81-100	.05	+	18	10%
101-120	0.1	+	16	20%

To Page 1

Witnessed & Understood by me,

Date

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Date

Deeracra Polans

2/16/95

Record d by

10.5 kb

Ig No. _____

to see: To try another mini prep - 10.5 kb fragment contained in pDELT A - and amplify.

used overnight cultures grown in the presence of Telt + Kao saved at 4°, over here and.

Alkaline lysis protocol. all resuspended in 2.5 ml of TE extracted from 3 x 1.5 ml culture

Did an enzyme titration }
at 2 μg " } - amplification done only with enzymes that don't have plasmids included was just 1 rx with Tag 0120

Vol. 50 μl.	200 μM dNTP	cycling 3 steps
4, 3, 2 mM Mg	(95°, 45")	95°, 3'
.4 μM primer	55°, 30"	25 cycles
{ 1, 2, 5 U enzyme }	72°, 5'	
{ .01, .02, .05 }		

repaired primex with buffer B containing 2 mM Mg

reduced supplement Mg accordingly. 2 mM 3 mM 4 mM

red 2 μl of miniprep template	0	5	10
con. consistency.	60	45	40

repaired 20x primex	50	50	300
x buffer 100)	1		
dNTP 20)			
P1 4)			
P2 4)			
temp. 40)			
enzyme (in 5 μl) 50)			
Mg (")			

5 μl / rx as needed.

enzyme 1 μl addin 5 + 2 = 7 take dry. conc.

To Pag No. _____

d & Understood by me,

Date

Inv nt d by

Dat

[Signature]

1/20/95

Recorded by

R. Stoenner

1/10/95

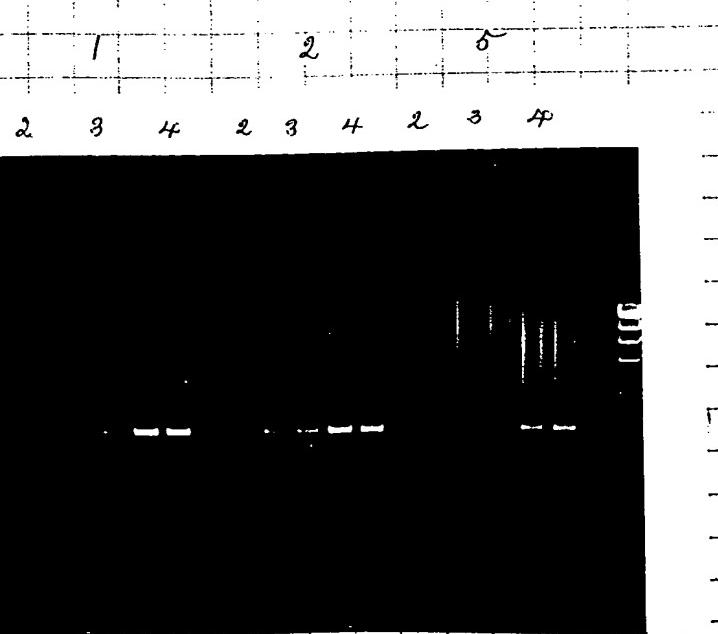
Project No. _____

Book No. _____

TITLE _____

From Page No. _____

1	2		2
3	4	10	3
5	6		4
7	8		2
9	10	2N	3
11	12		4
13	14		2
15	16	50	3
17	18		4
19	Taq	2N	2 ml



Result :

cycling has been optimized. - Job of sequencing
 2 mM Mg didn't work in any of the sets ? - It
 worked earlier in 25 μl as w Taq.

amount of template ?

Increasing the enzyme didn't seem to work
 no does Mg

Taq alone at 2N / 50 μl didn't work

- get fresh enzyme.

To Pg

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Dat

Inv nted by

Date

1/25/95

R corded by

Dr. Sivarajan

1/10/95

Stability study for PCK mix containing 1 unit of rTag

Exhibit 37
Appl. No. 09/558,421

B KN.

Assay date is
2-3-95

121

age No.		al enzyme added	To reaction	Taq units in mix	mix vol H2O/4
Experiments					
J. Solar of [1-20-95]	Rxn #				
2x R2G 0.1% TN (Tween/NP40)	1-3		2 (0.5%)	48 μl mix	= 48 μl mix
0.2% BJ	4-6		Tween 20/NP40	48 μl	so +2 λ Enzyme gives Vf = 50 μl
0.2% TX Triton	7-9				
0.01% TN	10-12		(-0.0004%)		
0.02% BSJ	13-15				
.02% TX	16-18		(.04%)		
1.0% TN	19-21				
2.0% BSJ Brij	22-24				
2.0% TX	25-27				
No detergent	28-30				
(1.1x)	31-33	3.64			
(5x) → Dilute $\frac{1}{2.5} = 0.4 \mu\text{l}/\text{μl}$	34-36	2			
2x R2G e 0.1% + Tag	37-39				
2x Tf 1 0.1%	40-42				
2x Verap. Buff	43-45				
5 μl	46-50	2			
dil = 0.04 μl/μl					

Taq in/
Taq TWS
31-5557
2x1 PSS, 7
buff

[10' 74°C, 10 μl 20 mM dNTP mix]
spot 40 μl on GFC new stocks

add dil buffer 0.9-20.94 (PSS)

Test Rxn mix	mix P.W.
EKBT 15 μl 1-31-95:	
no dil	2 48 μl
1/125	2
EP9 407	2
1/125	2

Made new mix with stock shown in red on P120 and repeated experiment on 2-3-95 - results on next page (P123)

incorporation!

Total 5 μl of #12 (5x) into 12 μl Tag dil buffer PSS, 7

To Page No.

signed & Understood by me, Janet Polcyn	Date 2/16/95	Invent'd by <i>J. Polcyn</i>	Date 2-1-95 2-3-95
		Recorded by	

Project No. _____

122

Book No. A10TITLE
UNI/ptRelative
to T_{0y}T_{0y} = 5u/ λ N
to T_{0y}

From P.

1	8410.00	} 8819	.037	.03
2	9136.00			
3	8912.00			
4	7465.00	} 7952	.033	.03
5	8664.00			
6	7728.00			
7	7737.00	} 7580	.032	.03
8	7235.00			
9	7769.00			
10	7579.00	} 6878	.029	.02
11	(3001)00			
12	6178.00			
13	7484.00	} 7812	.033	.03
14	7833.00			
15	8119.00			
16	6228.00	} 6566	.027	.02
17	6715.00			
18	6755.00			
19	8215.00	} 7824	.033	.03
20	8743.00			
21	6514.00			
22	7996.00	} 8413	.035	.03
23	8661.00			
24	8581.00			
25	7644.00	} 7533	.031	.03
26	6981.00			
27	7976.00			
28	4900.00	} 4989	.021	.02
29	4647.00			
30	5419.00			
31	7509.00	} 7702	.032	.03
32	6923.00			
33	8674.00			
34	8196.00	} 8075	.034	.03
35	7970.00			
36	8060.00			
37	8015.00	} 7442	.031	.03
38	7358.00			
39	6954.00			
40	8055.00	} 8479	.035	.035
41	8359.00			
42	9023.00			
43	7844.00	} 7811	.032	.03
44	7351.00			
45	7638.00			
46	9312.00	} 9580 (0.04)	.04	.04
47	9496.00			
48	9290.00			
49	9726.00	} 58661.00	2 μ l	by definition
50	10073.00			
utiv.	58661.00			
51	60427.00			
52	60427.00			

$$\text{ave} = 59544 \Rightarrow 1,428,600 \text{ cpm/50} \mu\text{l Rxn}$$

37.2 cpm/ μ mol

To Page No

Witnessed & Understood by me,

Deborah Polcino

Date

2/16/95

Invented by

Recorded by

[Signature]

Date

2-3-95

119/95

plates / 6.4, 8, 10.5, 20, 29 kb.

Page No. _____

Cope and Rogers
Teller

Purpose: To make more clearer plates of 6.4, 8, 10.5, 20, 29 kb.
 plated quite a few for each at diff. dilutions.

cultures

These are grown under stronger conditions in the presence
 of Tek + Kan.

Used Amp plates, typ. at 37° over night.

6.4, 8.0 - colonies were small, especially 6.4
 same each time whenever Klett. - didn't remember how
 well worked ok. - well spread out they were last time
 bigger colonies.

streak them all out 4°.

picked a few single colonies from 6.4 & 8.0
 grew them again for fresh minipreps. grown under
 Kan + Tek conditions.

From 8.0 - did minipreps by alkaline lysis method, extrapolated in
 $15 \text{ ml } 9 \text{ to } \times 12 = 180 \text{ ml Total.}$

6.4 kb streak at 4°.

To Page No. _____

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Date

1/10/95

Invented by

Recorded by

J. Sivanandan

Date

1/11/95

126

Project No. _____
Book No. _____

TITLE ^{32}P 23mer degradation reaction cond

(see P 80)

From Page No. tube # 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
wt

5X mixtes # 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 1 10

P. 125 μl → ✓ ✓ 44

$(0.65 \mu\text{mol}/\mu\text{l})$ 32P 23mer $0.267 \mu\text{mol}/\mu\text{l}$ primer
 $\times 0.6$ → ✓ - -

(run as P75)

Vent pol 2 μl → 22

0.01 $\mu\text{l}/\mu\text{l}$

Whist in Vent storage
in dilution buffer
from NED

50% glycerol 2.0 μl → ✓ ✓ 2.8

H₂O 13.4 → 10.6

Vf = 20

70 °C 30'

add 10 μl cycle seq stop

2.0 μl → ✓ ✓ 1.2

* 32P 23mp19 → 10 K λ
Vf 20°

(13K λ)

cocktail

(A)

(B)

preheat tubes to 70 °C

Then add Vent

for 30 min 12 min

32P 23

H₂O

50% glycerol

Vf = 18.2

add H₂O/tube # 1-10

and 23 which

gets 2 μl of

Vent dil buffer

7.8 λ

7.8 λ

36.4

137.8

174.2

182

11-20

0.02 unit Vent at 100000 U/mg

MW \approx 100000

$\Rightarrow \frac{1}{2} 0.1 \mu\text{mol pol}/1 \text{ unit}$

$= (0.002 \mu\text{mol pol total pol})$

$0.1 \mu\text{mol primer}$

$\frac{0.002 \mu\text{mol pol}}{0.1 \mu\text{mol primer}}$

(PO primer/pol)

$0.38 \mu\text{mol circles}/0.002 \mu\text{mol pol} = (192 \text{ circles/pol})$

* 32P 23 mp19

$(\approx 20\% \text{ with } 23 \text{ mp19 added})$

32P 23 6.267 $\mu\text{mol primer}/\lambda$ P75

m13 mp19 + 0.1 ml, 0.2 $\mu\text{g}/\lambda$

$= 0.084 \mu\text{mol circles}/\mu\text{l}$

0.1 μl .3

0.6 μl 1.8

7.3 27.9

15 μl 30

3.47 μl

2.05 μl

2.78 μl

50 °C, 5' cool slow

To Page No

Witnessed & Understood by me,

Deborah Polcari

Date

2/10/95

Invented by

[Signature]

Date

2-10-95

Recorded by

ig No. _____

1/15 Vast dil buffer

1/15 Vast dil buff

Vast dil buffer

50 mM Tris HCl pH 7.4

1 mM OTT

0.1% NP40, Tween 20 each

50% glycerol

10 mM K-EDTA

run 16% PAGE plus new reactions on 2-13-95

dilute 1/5
with 10mM

Tris HCl pH 7.5

20 mM

0.2 mM

0.2

10% glycerol

10 mM

16% PAGE see p 144,

24 25 26 27 28 29 30 31 32 33

vast buffer

2 2

2 .1

(1 P125)

4 4

✓

vast buffer)

4 4

4 ✓

vast buffer

2 2

2 ✓

Σ 20 mM

10 P125

4 4

✓

-Cherry wine)

en (P126) 0.6

-

✓

19 (P126 · 0.77 pmol cellulase/1

5 5 ✓

· 0.38 pmol circ

· 0.01 uA

total

0.1 uA

glycerol

5 5 ✓

15.4 15.4 13.4 13.4 8.6 8.6 13.4 13.4 11 4.2 ✓

if = 20 μl

2.8 ✓

my Cherry has one

70°C, 12'

2.8 ✓

10% glycerol at 1X

% PAGE

start 1700V at 1.45 min

40% Acrylamide 200g

at 2.5Watts, 15 mAmps

0.8% Bis

4%

H₂O

get ~ 7.4 cm/hr

need 7.0 more

so next 3 hr

500ml

went to 30 watt

constant set

2200-2250V

8.7 cm/hr

2200-2250V

To Pag. No.

8.7 cm/hr

To Pag. No.

space

space

space

big plate

Date

2/16/95

Invent'd by

2-10-95

Record'd by

2-13-95

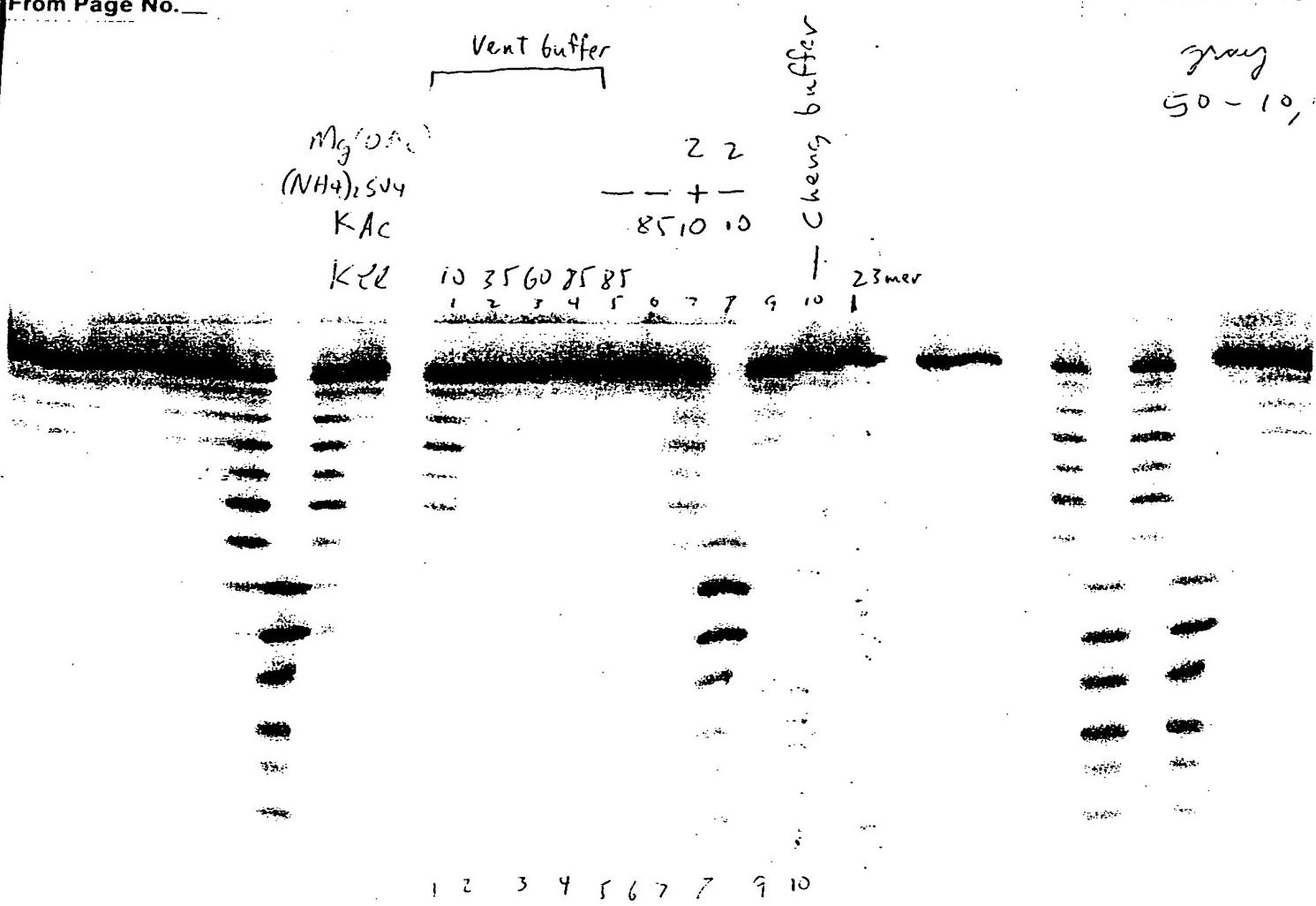
128

Project No. _____

Book No. _____

TITLE _____

From Page No. _____



Results:

- #1, 10 KCl + MgSO₄ is same as KAc, MgOAc - get degradation if K \leq 1
- 1 - 5 increasing ionic strength eliminates degradation. #5 also 85 mM KAc same as KCl 85 mM
- 8 leave out (NH₄)₂SO₄ get best result degradation of all
(don't have (-)(NH₄)₂SO₄ for 10 mM KCl and MgSO₄ only 85 mM)
this result also consistent with ionic strength effect
- 9 substitute tricine for Tris in Vent buffer has no effect
- 10 complete Cheng buffer - no degradation can be fully explained as due to 85 mM KAc - see # 4, 5 - 85 mM KCl or KAc to degradation in Vent buffer

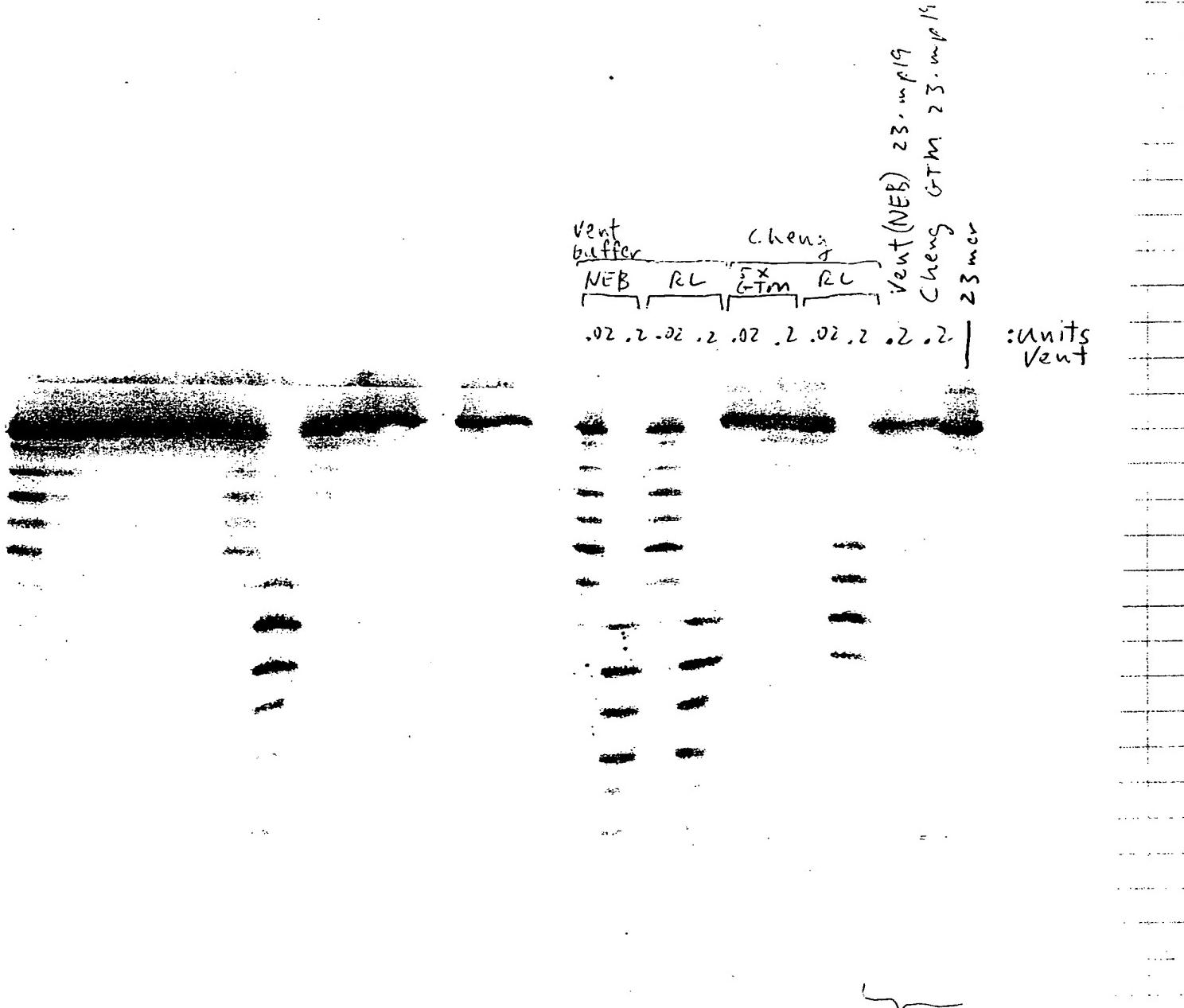
Witnessed & Understood by me,	Date	Invented by	Date	T Pag
Deborah S. Polkay	2/16/95	K. J. Polkay	2-13-95	

- 02/14/95 - 06:45 pm

1.00x Counts

49.97

10000.00 D



To Page No. _____

sed & Understo d by m , see a Polar	Date 2/16/95	Invented by 	Date
		Recorded by	

130

Tne vs Tag

Project No.

Book No.

TITLE

effect of K_{cl} on pol on M13
and primer degradation

From Page No. —

K_{Cl} mM

(1) (2) (3) (4) (5) (6)

50 75 100 50 75 100

pol —————→

4 8 4 8

✓ ✓

✓ ✓

pol —————→

(0.64 pmol)

1.6 µl —————→

✓ ✓

2.0

2.4 µl —————→

✓ ✓

1.5

2 —————→

✓ ✓

0.0

10 X Tag PCR buff

K_{Cl} 0.5 mM

^{32}P 23-mp19 ($0.64 \text{ pmol } ^{23}\text{-mp19}$)

1.2 mM 4dNTPs

MgCl₂ 50 mM

Tag 0.4 uM

Tne 0.8 uM

H₂O

58 54 50 58 54 50

✓ ✓

0.2

prefet tube

✓ = 10 µl

to 70°C, start with 2 µl pol

remove 10 µl at 1, 2, 5, 10 min to 5 µl cycle seq stop

* rTag EKBT1 1-3195 5 uM
Tne 5 uM A. Goldstein

{ both diluted in Tag dil buffer

^{32}P 23 mer names as P.75 (0.267 pmol 23mer/uL)

^{32}P 23-mp19

^{32}P 23-mp19 0.267 pmol 23mer/uL

15.8 µl

(4.2 pmol 23mer/uL)

M13 mp19 0.2 µg/uL
(0.084 pmol circles/uL)

50 µl

(4.2 pmol circle/uL)

1 MTMS 7.5

0.6

6.6 µl

0.064 pmol

23-

use 1 µl / 10 µl re

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Deacon at Stamps

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2/16/95

Inv nted by

Re ord d by

Date

2-15-95

To Page 1

= River degradation
(see PFO)

Project No. _____

Book No. _____

131

15-17 28-30 31-33 34-36 37-39 40

ag N.	(7)	(8)	(9)	(10)	(11)	(12)	?	
vent	80	8*			80	80	✓ Does DMSO have any? ✓ no contamination?	
Taq PCR buffer, DMSO		1.6	80	80	80	1.6	✓ C = 2% DMSO	
23 min	1.91						✓ ($0.64 \text{ pmol } 23 \text{ min} / 10 \mu\text{l} = 6.4 \text{ nmol}$) Primer	
1/2 50 mM MgCl ₂	—	—	2.4	—	—	—	✓ (note Vent buffer has 2 mM MgCl ₂)	
0.7 uM	2		4	8	—	—	✓	
0	66	64.4	69					
	63.7	59.7	55.7	66.5			✓	
heat to 70°C, remove 10 µl at					2, 1, 1			
pol. circles							15 min only tube 4	
0.1 unit Taq = $\frac{0.005}{0.01} \text{ pmol}$ (per 10 µl PCR)								
0.064 pmol 23 min (per) (= 0.464 nmol total / 10 µl)								
pmol circle								
pmol pol	0.012							
							3 ends / pol molecule	

Expected results

$\frac{0.1 \text{ u}}{10 \mu\text{l}}$ Taq gives 1 nmol at 1/30'

have 0.464 nmol at $\frac{1}{10} \text{ reaction volume}$

$\frac{0.064}{0.012} = 5.3$ min to replicate all DNA at least twice
based on units - (but not sure M13 gives same units)
1 min would be ≤ 500 at extension at unit initial rate

composed by PCR

1. This would be 0.5 units / 5 µl PCR

2. 6.4 nM primer (so $10 \times$ less than 100 nM primer).

T Pag No. _____

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erica Polany

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2/16/95 Inventor by
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Date

2-15-95

Project No. _____

Book No. _____

TITLE _____

From Page No. ____

100 b.p. ladder cat 10072-015

10 μ l H₂O (vortex)

1 μ l 10 mM Cu/10 μ l Pd/CHP

15' 37°C \rightarrow 10 λ 0.2 mM EDTA

get total $> 10^7$ cpm

load 0.2 μ l 10^7 cpm \Rightarrow 5000 cpm/ μ l
 $(20 \lambda \text{ total}) (2.0 \text{ bands})$
apth 10 μ l EDTA

part 20 μ l (Rxn + EDTA)
10 μ l cycles seq stop
30 μ l $\geq 300,000$ CPM/ μ l
10,000 CPM/panel/ μ l

load 1 μ l

To Page N

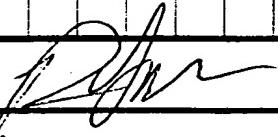
Witnessed & Understood by me,

Deececa Polens

Date

3/16/95

Invent'd by



Date

2-18-95

Record'd by

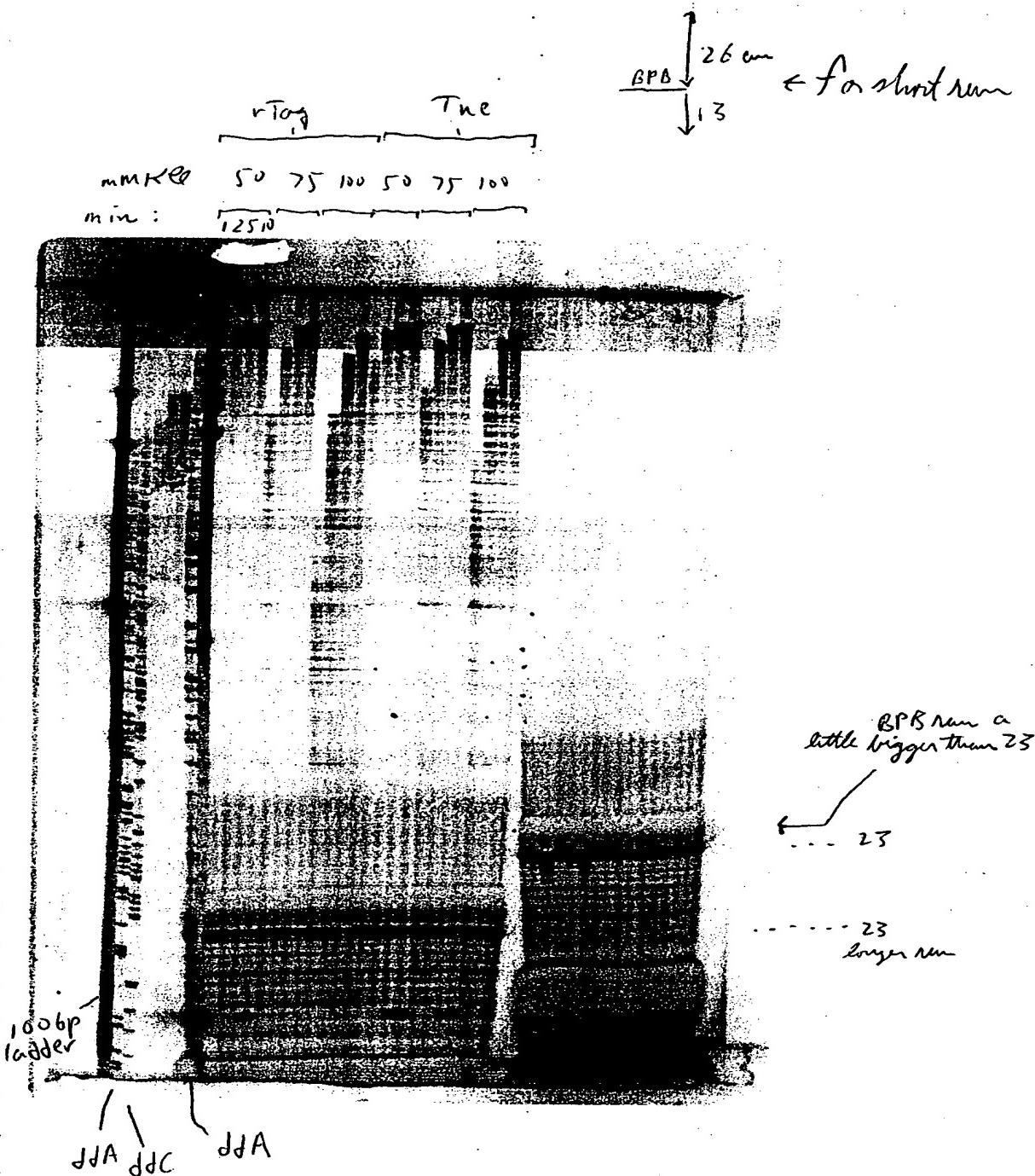
134

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Book No. _____

TITLE _____

From Page No. _____



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Date

R cord'd by

2-17-85

Deevee A. Polansky

3/16/95

136

Project No. _____

Book No. _____

TITLE _____

From Pag No. _____

primer 5'60826 (23mer with terminal A instead
of G called "AC")

74.6 nmol total
 $74.6 \times H_2O$

$C_f = 100 \text{ pmol primer / \mu l} (= 100 \mu M \text{ prim})$

Kinase

(100 μM primer)

23mer "AC" 100 pmol 23mer /

5X Kinase buffer

^{32}P ATP 10 mCi / μl (3.3 μM ATP)

PNK 1 u / μl

H₂O

2 μl ✓ ✓ 200 μ
8 ✓ ✓ 23-
20 ✓ ✓ 66 μ
2 ✓ ✓

30' 37°C \rightarrow 60°C 5'

40 μl CP = 5 μ

use 2 μl / 50 μl PCR
for 200 nM primer

note 1 unit T4 kinase converts 1 nmol ATP / 30' at 37

To Page N

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Debrae Polley

Date

3/16/95

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Date

2-20-95

Primer degradation test under PCR conditions (200 nM primers > 2 uM
and Δ Kell) Prj CrN

Bo k N .

Exhibit 45

Appl. No. 09/558,421

137

ge N .

1 2 3 4 5 6 7 8 9 10 11 12

x Tag PCR buff

5 μ l — → ✓

"AC" 1 p136 (1 μ M)

2 μ l — → ✓ (Cf = 0.2 μ M 23u)

200.5 M

- 1 2 3 4 5 - 1 2 3 4 5 /

ne 5 u/l →

2 μ l — →

dilute to 2 u/l

(4 units total)

T, T₀/K 20 $^{\circ}$ A

(0.4 units)

stata two.2u/l →

2 μ l — →

MgCl₂ 2.50 mM

1.5 μ l — → ✓

H₂O

1.5 μ l — → ✓ (Cf = 1.5 mM)

$$V_f = \frac{39.538}{50 \mu\text{l}}$$

MM Kell Cf = 50 60 70 70 80 100 50 60 70 70 80 100

70 $^{\circ}$ C, remove 10 μ l to 5 μ l stop at 20, 60, 120 min

Results on PIST

To Page No.

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Date

several Bolups

30

6/95

Recorded by

2-21-95

138

Project No. _____

Book No. _____

TITLE 33 mers mismatched and mismatched

From Pag No. _____

11.64 nmol "33 correct"
(primer # 538; DG1 (G01))

582 μ l H₂O

has correct G at 3' end at 1
of 8 sites in MCS G → 3'
GAATTC

\Rightarrow 20 μ M primer

13.42 nmol
33 mismatched

671 μ l H₂O

\Rightarrow 20 μ M primer

33 correct 1 μ M $\times \sqrt{5.3}$ ✓ ✓ (5.3 pmol total)
(1 pmol / μ l)

33 mismatch 1 μ M
(1 pmol / μ l) 5.3 ✓ ✓

(1.67 μ M ATP)
YATP 10 mCi/ml X✓ 4 4 ✓ ✓ ✓ ✓ (6.68 pmol)
5X Klenow buffer X✓ 4 4 ✓ ✓ ✓ ✓
PNK 1 nM ✓ 1 1 ✓ ✓ ✓ ✓
H₂O X✓ 5.7 5.7 ✓ ✓ ✓ ✓
 $\frac{20 \mu\text{l}}{20 \mu\text{l}}$ 37°C, 30' \Rightarrow 5'

1.10 pmol circle) \rightarrow m p19 0.25 ug/l 1.1 x 36.7 36.7 0.1 ✓
1 M Tris pH 7.5 X 0.6 ✓
H₂O ✓ X $\frac{20 \mu\text{l}}{6.6 \mu\text{l}}$ ✓
VF = 6.6 6.6 μl
5', 95°C cool slow

CP
0.3 pmol
Primer
0.6 pm
Circle
circle/
= 2
exce

use 2 λ / 20 μ l reaction

= 0.6 pmol primer in 20 μ l

To Page 1

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Deborah Polanyi

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Invented by

[Signature]
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Date

2-23-95

GEL

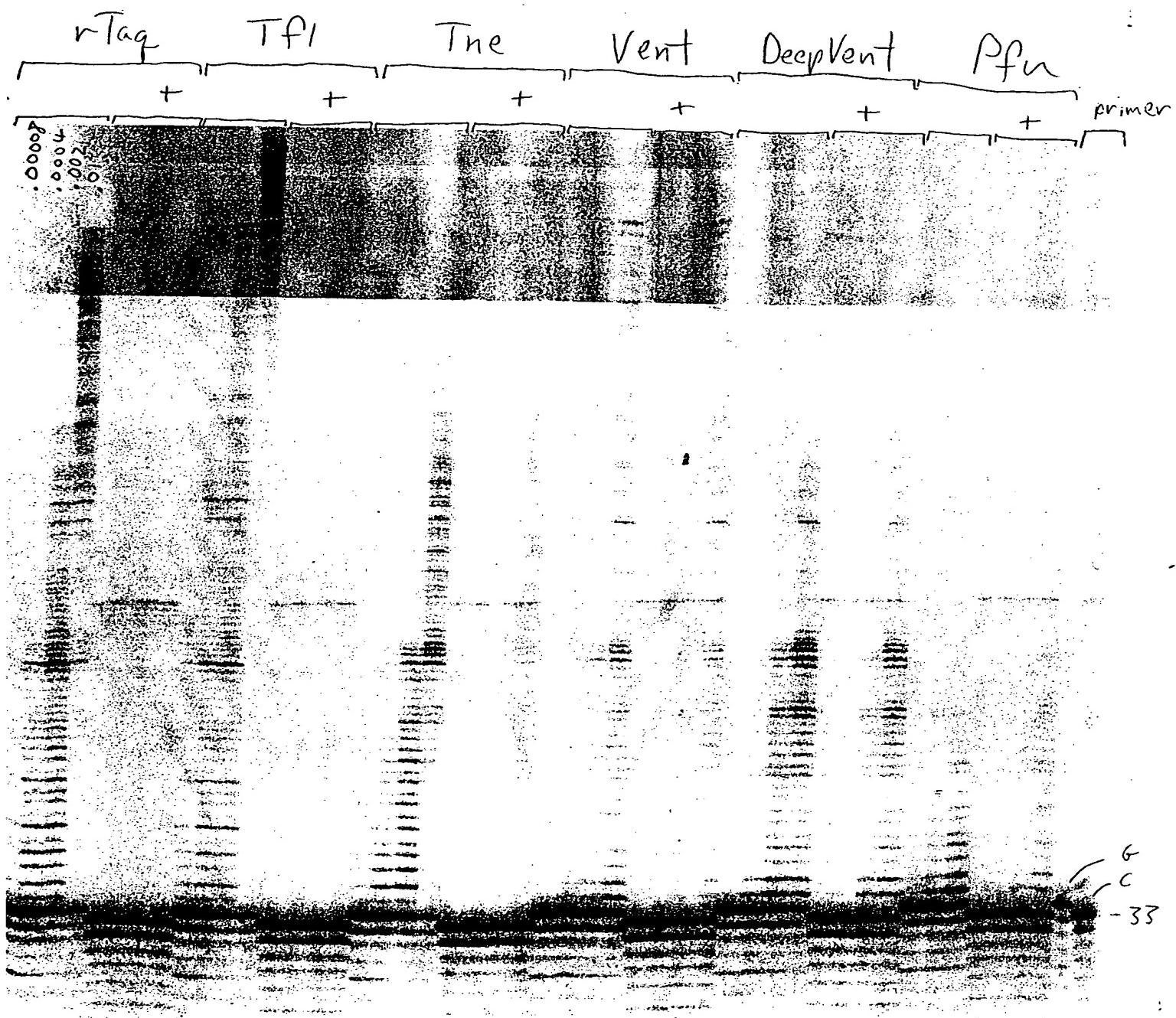
- 02/24/95 - 01:10 pm

0.66x Counts

0.11 [REDACTED] 2000.25 D

Exhibit 47

Appl. No. 09/558,421



33 C
— C —

— C — G

circles/mol molec

56

282

1410

7050

O.GEL

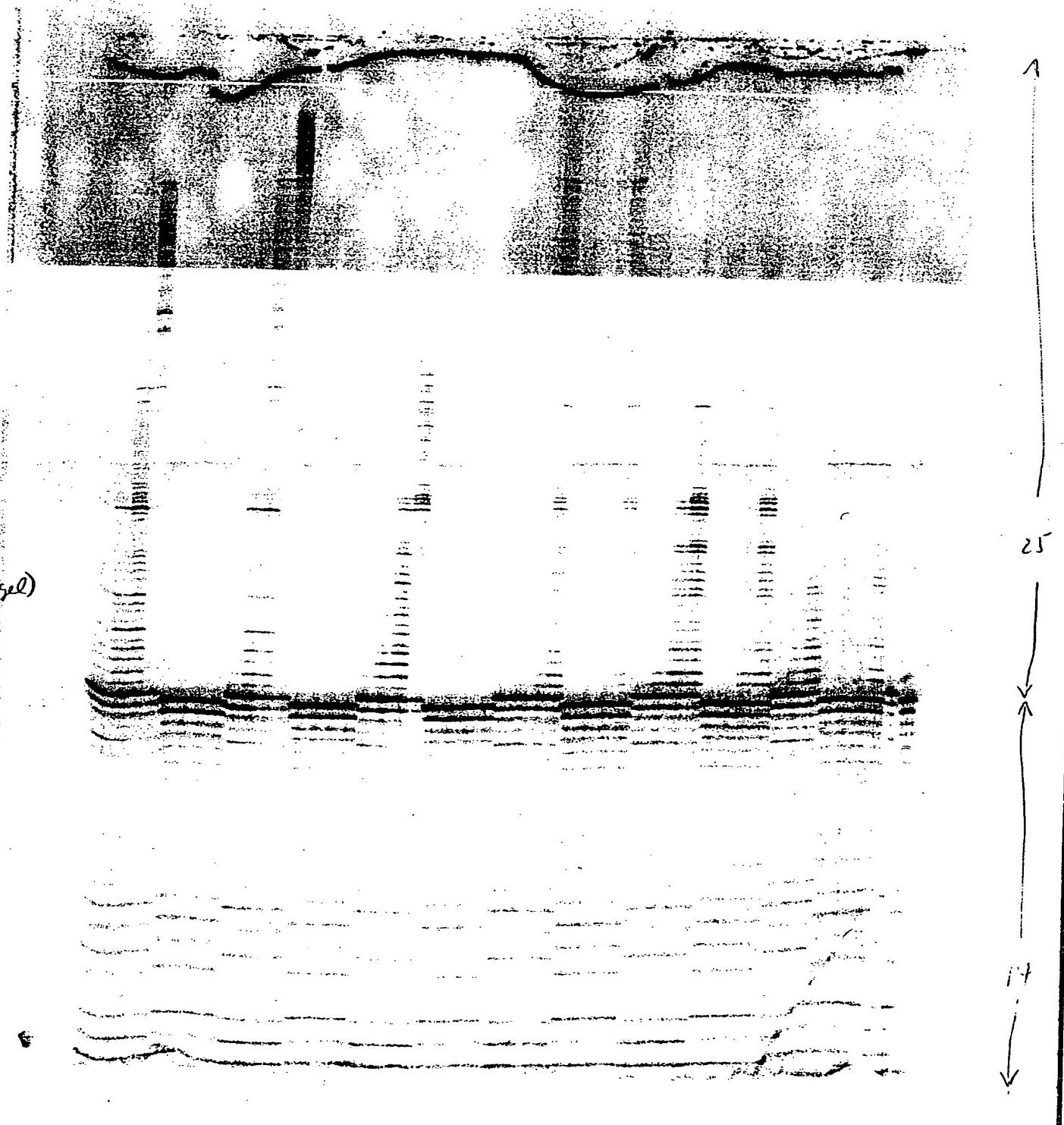
- 02/24/95 - 01:10 pm

0.50x Counts

0.11

2000.25 D

16% PAGE 33 watts, 4½ hr
XC went 21.7 cm ($R_f = \frac{21.7}{39} = 0.56$)
33 mer migrated 25 cm
XC runs as a 40 mer



could run XC to ~ 30 cm

P
e. 33 mer went
25 cm of 39 cm
gel by the

Pr ject N _____

Book No. _____

139

ag N _____

To Page No. _____

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erica Polans

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Date

2-24-95

Project No.

140

Book No.

TITLE

From Page No.

109

TfI

The

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

^{32}P Correct - mp19 (p.138) 2 → 2 → 2 → 2 →

^{32}P mix - mp19 (p.138) 2 → 2 → 2 → 2 →

10 mM dNTPs each

0.4

50 mM MgCl₂

0.6

10X PCR buffer

2

10X Vent buffer

2

10X Pfu buffer

14) →

H₂O

rTaq .00008 u/l

1

-31-95 .0004

1

.002

1

.01

1

TfI .00007

1

picomolar .0004

1

150000A .002

1

.01

1

The .00007

1

.0004

1

.002

1

.01

1

Vent .00008

1

.0004

1

.002

1

.01

1

Depot A .00008

1

.0004

1

.002

1

.01

1

Pfu .00008

1

.0004

1

.002

1

To Page 1

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Deborah Robins

Date

3/16/95

Invented by

Recorded by

Date

DD-2455

Project No. _____
Bk No. _____

Routine
cocktails

141

Fig N.	Vent	Deep Vent	P/Pn	10% succinic acid	10% succinic acid	10% succinic acid	10% succinic acid														
27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48

→	2 →	2 →	2 →	2 →	✓	28	20	1L
---	-----	-----	-----	-----	---	----	----	----

2 →	2 →	2 →	2 →	✓	✓	5.6	5.6	4	4.2	4.2
-----	-----	-----	-----	---	---	-----	-----	---	-----	-----

✓ 3.4 3.4 - - -

✓ 28 28

✓ 20 20

2 →	✓	12	12							
14 →	✓	196	196	196	146	146	146	146	146	146
		14 Pm	150	150	150	150	150	150	150	150
		14 Pm	196	196	196	196	196	196	196	196
		201 Pm	60	60	60	60	60	60	60	60

2 min 70°C
add with 10 μl capping stopp

note: 0.03 pmol 3' ends / Rxn
for .00008 units (lowest level):

$$(10 \times 10^9 \text{ nmol/unit}) (.00008 \mu\text{l}) = .03 \text{ pmol}$$

30 min

∴ expect only ~ 1 nt addition for
each primer in 1 min (base
on even distribution and provision
of 1).

To Page No. _____

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ceresia Polcino

Date

3/16/95

Initiated by

Recorded by

Date

12-24-95

142

Project No. _____

Book No. _____

TITLE

Repair of 3' mismatch

for Tf1 ± Vent and rTaq ± DV, Pfu T.

From Page No. _____

1 2 3 4 5 6 7 8 9 10 11 12 13 14/5 16 17 18 19 20 21 22

32 P33 mis-mP19

2 -

(P138 evnt 0.01 fm primers/1)

10 mM dNTPs

0.4

5X Cheung complete (4mM) ✓4

5X elongar

H₂O

✓ 13 12 → 13 12 → 13 → 3,4,12,4 → 13,4,12,4 → 12,6 →

Tf1 0.1 u/l

✓ 1 1 →

Vent .002 u/l

✓ 1 1 →

.01 u/l

✓ 1 1 1 1

.05 u/l

✓ 1 1 1 1

rTaq 0.5 u/l

5 u/l

0.2 →

0.2 →

Deep Vent .002 u/l

.04, .005

.05

1 1 1

1 1 1

1 1 1 1

Pfu .002, .005

.04, .005

.05

Tne .002, .01

.04, .05

.05, .01

VF-201

Mix all reaction to 70°C, start by addition of
3'P33 mis-mP19, add 10 µl cyclase stop at 2 minutesrTaq, The Tf1 use Taq dil buffer
Pfu, Vent, Deep Vent are NEB Vent dil buffer

To Page N

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Deborah Bolano

Date

3/16/95

Inv nt d by

Ric

Date

2-27-95

Rec rd d by

27	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----

>

low wtag

High

Mr
tag

--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

>

✓

6

6

6

3.8	12.4	→	12.6	→	13.4	12.4	→	13.4	12.4	→	12.6	→			
-----	------	---	------	---	------	------	---	------	------	---	------	---	--	--	--

v	60	60	60
v	186	186	189

1.2	→	0.2	→	0.2	→										
-----	---	-----	---	-----	---	--	--	--	--	--	--	--	--	--	--

v	3	3	—
v	255	255	215

use 7 μ l/Rxn	"	"
in 12-15	16-19	20-22
23-26	27-30	31-33
34-37	38-41	42-44

12, 23, 34 used
1 μ l H₂O added
Very old
buffer

16% PAGE
start 11:12 PM
50 watts (2400V)
for 1 hr, 20 min
then 40 watts at 12:30

* 8 μ l 320
35 min (P188)

36.7 μ l in p19 0.26 μ g / l

1.8 (mTris pH 7.5)

151.5 H₂O

(0.01 pmol/ul and 0.02 pmol/ul)

(~5 pmol each circle)

To Page No. _____

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Date

Invented by

Date

Suzanne Polcino

3

/ 16/95

Recorded by

2-27-95

146

Project No. _____
Book No. _____

TITLE ΔKAc . Effect on pol ac exo, Tne.

From Pag No. _____

1 2 3 4 5 6 7 7 9 10 11 12 13 14 15 16 17 18 19 20

$5 \times$ Mergs (no KAc , no $BuNSO$, no Glycerol)
(at $5 \times = 100$ mM Tricine pH 8.7,
5 mM $Mg(OAc)_2$)

V 4 -

KAc 0.2 M

V 0 1 2 3 4 5 6 7 8 9 10 0 1 2 3 4 5 6 7 8

X
33 correct. mp 19 (same as
P138, 0.06 pmol circle/λ)

V 2 -

^{32}P 33 correct 5 μM primer
(as was done for "Ac" on 136)

10 mM $4JNTP_2$

H₂O

V 0.4 -

v Tag 0.001 u/λ

2 -

2 -

Tne 0.004 u/λ

Vf = 20 λ

70°C, 5'

X 33 correct has
same 5' end as 23mer sequencing primer

To Page N

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Date

Invented by

Date

Deborah W. Polkinghorne

3/16/95

Recorded by

3-1-95

26 27 28 29 30 31 32 33

→ ✓

2 3 4 5 6 7 8 9 10 ✓

JX Chenz on P 79:

20 mM Tris HCl pH 7

1.2 mM MgCl₂

8% glycerol

2% DMSO

plus KCl which is varied
from 0-100 mM in
this experiment.

↓ → ✓

2 2 2 2 2 2 2 2 2 2
= = = = = = = = = =



→

✓

70°C, 60'

start 11.18

To Page No. _____

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enacted Polaris

Date

3/16/95

Invented by

Koh
Recorded by

Date

3-1-95

Project No. _____

Book No. _____

TITLE 10X PCR same as P140
is Tne inhibitory at 7 units?

148

From Page No. _____

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

10X Tag PCR buffer ✓ 2

? 2nd P33 correct: mp19 (P146)
(sub 138)

✓ 0 mM MgCl₂

1.0 mM DNTTPs

H₂O

✓ 0.6

✓ 0.4

✓ 14

→ 13 → 14

r Tag: ✓ . . .

0.25 u/l

0.5 u/l

✓ 1

✓ 1

✓ 4

✓ .6

✓ .8

✓ 1 → ✓

Tne

0.25 u/l

0.5

1

✓ -1 ✓ 1

✓ 1 ✓ 1

✓ 1 ✓ 1

✓ .4

✓ 2

✓ 3

✓ 4

✓ 5

✓ 4

✓ .6

✓ .6

Tag Storage buffer

✓ 20 µl

✓ .6 .4 .2

✓ .6 .4 .2

✓ .6 .4

preheat to 70°C, add 2 µl 33-mp19 for 30 sec
kill with 10 µl 1 cycle rig stop solution
with 1.0 mM ultra EDTA → 50°C f = 2.0 mM EDTA in stop.

To Page No. _____

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Doreen A. Polans

Date

3/16/95

Invented by

Recorded by

Date

3-3-55

Project No. _____

Book No. _____

149

ig N .

To Page No. _____

d & Understood by me,

area Polaris

Date

3

Invented by

Recorded by

Date

16/95

150

Project No. _____
Book No. _____

TITLE AatII #1

³⁵P Kinaid

From Page No. _____

see P136 for ↑ [primer]

10 μM AatII #1

5x Kinaid
³²P ATP 10 μCi/μl
PNK 1 u/l

5 ✓
4 ✓
3 ✓
1 ✓
2 μl

(33 pmol ATP)

at 1X
5 mM MgCl₂ 55 μM
70 mM KCl

(⇒ now its 2.5 μM prime)

37°C, 30' → 80°C, 5'

↓ mix back into cold primer
at 5 cold to 1 hot primer

³²P AatII #1 2.5 μM 13.3 20 (2.5 μM)

cold AatII #1 16.6 2.5 (10 μM)

30 45 μl (6.67 μM) ($MgCl_2 = 2.2$)

use 1.5 μl / 50 μl PCR for 200 nM
(adds 0.067 mM $MgCl_2$ to PCR mix)

Ayoub R. used in 14 PCRs

remove 10 μl from each PCR to 5 μl stop cap
and store at -20°C over weekend.

Result: Ayoub R
did PCRs with 1 μl

note smear (see EtBr stain
(P136 photo)) is not hot:

so primer ("AatII #1") is not
needed for smear

To Page

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Deborah Polley

Date

3/16/97

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3-3-95

Pr j ct N _____

Book No. _____

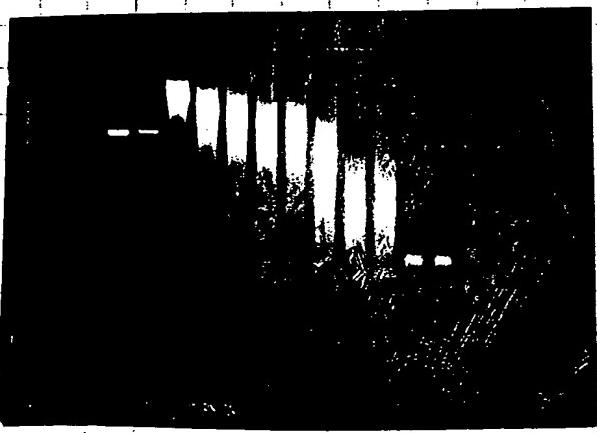
151

ig No. _____

106 ayawri 180 V
1 2 3 4 5 6 7 8 9 10 11 12
1 1 2 3 3 4 4 5 6 6 7 7

0.5 x TBE

(lot 2nd, 5 day)



To Page No. _____

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Date

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Date

asael Polans

3/16/95

Recorded by

3-7-95

Project No. _____

Book No. _____

TITLE

Unit assay for stability of rTaq in
PCR mix. Repeat of assay on P 121

152

From Pag. No. _____

This assay is 33 days after the first assay of 2-3-95.

Carry out all assays with exact same procedure
of P 120 - 122, same MgCl₂, TAPS, KCl mix of P 120
same stock of activated DNA, same 5' u/pl rTaq stock
in P 121 → (of 1-31-95) (3'P dGTP in a new stock of 10 mCi/ml on 3-12-95)

To Page N

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Devereena Boland

Date

3/16/95

Entered by

Recorded by

Date

3-9-95
3-8-95

related to Tag \Rightarrow u/
and

Project No. _____

Bk No. W1024

153

				activity	
1	23165.00				
2	26508.00	} 24896	.031	.037	84
3	25014.00				
4	24738.00				
5	23608.00	} 24616	.031	.033	94
6	25502.00				
7	23947.00				
8	24449.00	} 23577	.030	.032	94
9	22336.00				
10	19450.00				
11	20001.00	} 19701	.025	.029	86
12	19953.00				
13	21103.00				
14	20211.00	} 22157	.028	.033	85
15	25159.00				
16	19309.00				
17	18318.00	} 18853	.024	.027	89
18	18933.00				
19	22404.00				
20	25483.00	} 25532	.029	.035	87
21	22108.00				
22	20542.00				
23	27602.00	} 25507	.029	.035	83
24	21776.00				
25	22624.00				
26	23813.00	} 22151	.028	.031	90
27	20017.00				
28	10829.00				
29	12483.00	} 11703	.015	.021	(70)
30	11798.00				
31	23967.00				
32	25056.00	} 24527	.031	.032	97
33	24557.00				
34	26587.00				
35	23432.00	} 25000	.032	.034	93
36	24980.00				
37	25401.00				
38	24104.00	} 24694	.031	.031	100
39	24576.00				
40	25123.00				
41	25545.00	} 25962	.033	.035	94
42	27217.00				
43	24143.00				
44	23491.00	} 23703	.030	.032	93
45	23474.00				
46	30440.00				
47	31721.00	} 31731	(.04)		
48	30572.00				
49	32938.00				
50	32985.00				
51	17357.00				
52	17994.00	} 17377	.022		
53	16781.00				
54	144943.00				
55	145358.00				

Note #10 is for Alkylate. It will be
at 1% between 20/NP4 back in Reaction mix

To Page No. _____

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Enclosed Polar

Date 10/

16/90

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Ritter

Date

3-9-85

First off rule to use 1/60 Tag dil
between 20-40 min after mixing

Pr j ct N _____
B ok N _____

Exhibit 53
Appl. No. 09/558,421

155

Fig N Standard Tag units array as per 120-120

10 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

c# 1,23 4 5 6 7 8 9
tube# 1,23 4 5 6

21 22 23 24 25 2/27 28 29 30 31 32 33 34 35 36 37 38 39 40

10 11 12 13
7 8 9 10 11 12 13

41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 70 2hr 3hr

14 15 16 17,18
‡ 14 15 16 17

use a standard 1/60 dil^o

1797 ¹µl Tag dil buffer
3 ²µl ⁵ u/l Tag
Vortex 5"

use immediately in triplicate for reactions 1, 2, 3 at
0, 20 sec and 60 sec ^{1:45} ⁴⁰ ⁴⁵
also # ~~179~~ 20 ²² sit on ice + 2 3 hr before 101
EDTA (if no time at 74°C to see if any activity at 0°C.

To Page No. _____

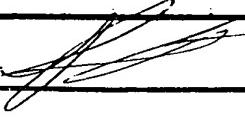
Read & Understood by me,



Date

3/16/95

Invented by


Recorded by

Date

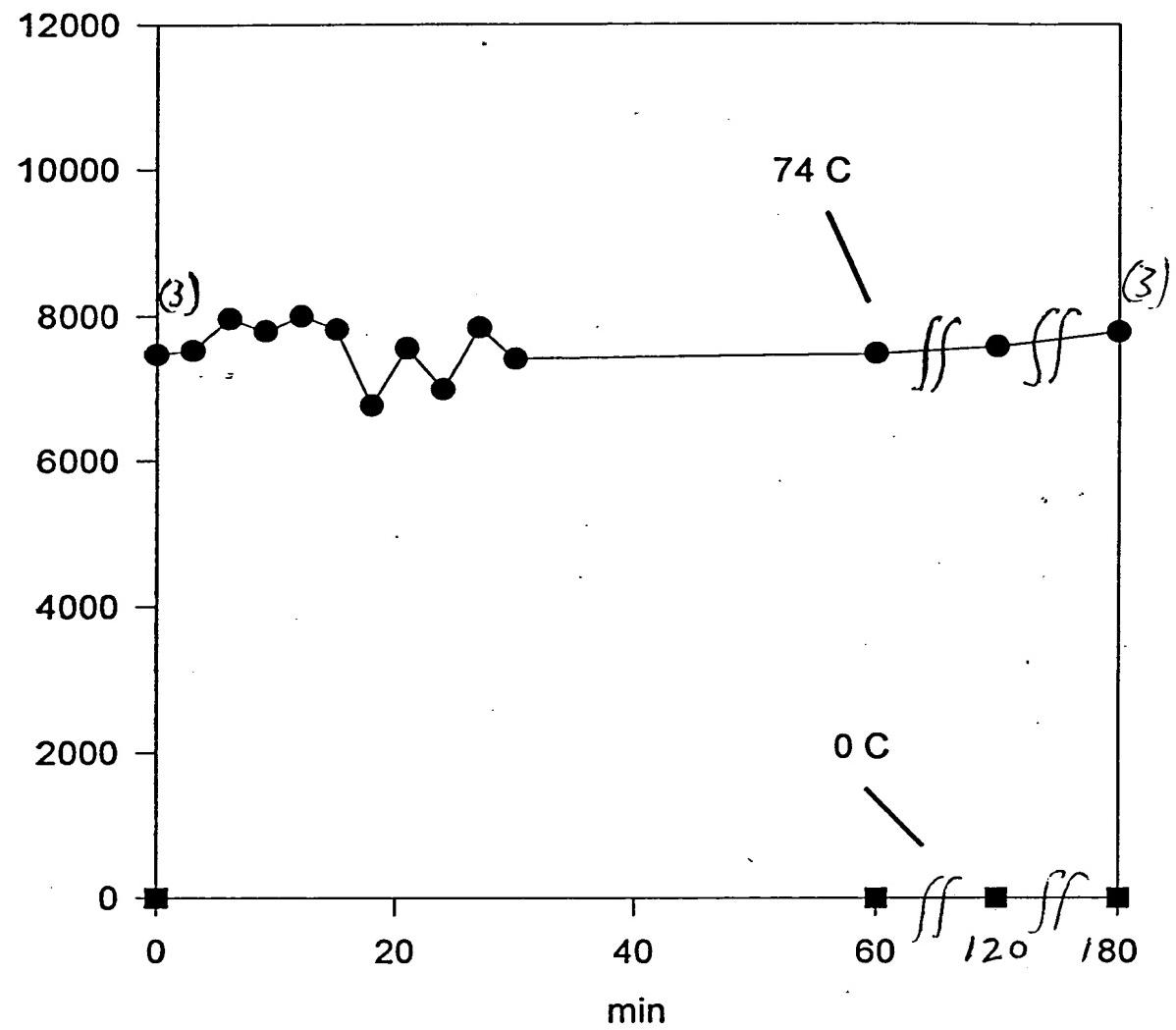
3/15/95

Project No. _____

Book No. _____

TITLE _____

Time allowed before assay of Taq dilution



SAM	CPM
1	738
2	845
3	705
4	770
5	809
6	802
7	820
8	796
9	692
10	764
11	733
12	801
13	760
14	970
15	765
16	784
17	750
18	828
19	827
20	27
21	66
22	40
23	59

to Page N.

I have read & understood by me,

Susan Polansky

Date

4/4/95

Inventoried by

[Signature]

Date

3-15-95

Tet stock / streak clones

Project N _____
Book N _____

Exhibit 54
Appl. No. 09/558,421

157

0.4g Tetracycline Sigma crystalline (not β -1 salt)
40 ml ETOH

Amp / Tet plates

have BBL Amp plates ($100\mu\text{g}/\text{ml}$) Vol x 15 ml agar
to make $50\mu\text{g}/\text{ml}$ Tet spread

Tet $10\mu\text{g}/\text{ml}$ Tet over夜 - let sink in >30 min

$50\mu\text{g}/\text{ml}$ Tet over夜 15 ml agar in plate

streak out cell (glyceral) streak of AR.

SUP 94-95 for streak names

grow at 35°C O/N

start 2 ml O/N from single colonies 3-21-95
of each in will grow, $100\mu\text{g}/\text{ml}$ Amp, $50\mu\text{g}/\text{ml}$ Tet

3-22-95

inoculate 0.4 ml of each O/N until 3.5 ml will grow
+ $100\mu\text{g}/\text{ml}$ Amp, $50\mu\text{g}/\text{ml}$ Tet

shake at 35°C starting at 8:30

To Page No.

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Date

Invent'd by

Barbara Polans

4/4/95

Recorded by

Date

3-20-95

3-21-95

Tet stock/^{PS} streak T^{r1}
clones

Exhibit 55
Project No. _____ Appl. No. 09/558,421
Book No. _____

157

age N

0.4g

Tetracycline

Sigma crystalline

(not B.C.
salt)

40 ml

ETOH

Amp/1st plates

have BBL Amp plates (100µg/ml) Voln 1ml aga

To make 50 µg/ml Tet spread

15 ml. 10 mg/ml Tet on each let sink in. ≥ 50 min

50 µg/ml Tet in 15 ml agarose plate

streak out cell (glyceral). streak of AR.

see P 94-95 for stock names

grow at 30°C O/N

start 2 ml O/N from single colonies 3.21-95
of each in and grow, 100 µg/ml Amp, 50 µg/ml Tet

3.22-95

immediately 0.4 ml of each O/N into 35 ml with 50 µg/ml
+ 100 µg/ml Amp, 50 µg/ml Tet

Mix at 30°C starting at D.5

To Pag No.

Used & Understood by me,

Karen Polans

Date

4/4/95

Invented by

Recorded by

Date

3-20-95
3-21-95

Tet stock / streak T^r clones

Project No. _____
Book No. _____

Exhibit 56
Appl. No. 09/558,421

157

age N

0.4g Tetragaine
40 ml ETOH
Signor crystalline (hot & cold salt)

Amp/Tet plates

have BSC Amp plates (10⁵µg/ml) Vol x 15ml agar
to make 50µg/ml Tet spread

T50µl 10mg/ml Tet on each - let sink in ≥ 30 min
50µg/ml Tet in 15ml agar in plate

streak out cell (glyceral) streaks of PCR.

see P 94-95 for streak names

grow at 35°C O/N

start 2ml O/N from single colonies 3-21-95
of each in will grow, 100µg/ml Amp, 50µg/ml Tet

3-22-95

inoculate 0.4ml of each O/N into 35ml will grow
+ 100µg/ml Amp, 50µg/ml Tet

shake at 35°C starting at 3-23

T Page N

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Date

Invent d by

Date

Barbara Polans

4/4/95

Record d by

3-20-95

3-21-95

From Page No. _____

3-22

30°C Assay

Start 8:30

12:30

• 274

2:00

• 770

\downarrow 42°C , 15 min

\downarrow 1 hr 37°C

extract and 55°C heat for Fr I is
same as p 95 and P115, 6

3-23

pol assay is same as P95 except add just

$2 \mu\text{l}$ 'Fr I' / $97 \mu\text{l}$ Rxn cocktail

and remove time points

assay 1 2 5 μl of ~~ben~~ Fr I' in $50 \mu\text{l}$

Taq unit assay (using TFI buffer system)

1 for 5 $\frac{1}{2}$ min at 74°C

3-23-95

12-15-94

%

106 (100) 64

107 H 87 92

108 H 86 (100)

152 P3 59

151 56 95

202 20 26

109 2 11

To Page No. _____

Witnessed & Understood by me,

Dat

Invented by

Date

Deanne a Polcyn

4/4/95

Rec rd d by

3-23-95

Project No. _____

Book No. _____

TITLE _____

158

From Page No. _____

3-22.

30°C
Start 8:30

A550

12:30 .274
2:00 .770

$\downarrow 42^{\circ}\text{C}$, 15 min

\downarrow 1 hr 37°C

extract and 55°C heat for Fr I is
same as p 95 and p 115, 6

3-23

pol assay is same as P95 except add just
~~2 μl Fr I / 9 μl Rxn cocktail~~

and remove time points

assay 1, 2, 5 μl of ~~Fr I~~ Fr I' in 50 μl
Taq unit assay (using TF1 buffer system
for 5 $\frac{1}{2}$ min at 74°C)

3-23-95 12-15-94

%

106 (100) 64

107 H 87 92

108 H 86 (100)

152 P3 59

151 56 95

202 20 26

109 2 11

To Page No. _____

Witnessed & Understood by me,

Deborah Polkay

Date

4/4/95

Invented by

Recorded by

Date

3-23-95

Repeat of 1971-71: 14'1 clones
except: induce 42° 15' → then 37°C

Project N . _____
Book No. _____

Result:
get n10X
more activity
if 37° instead
of 42°C after
the 42°C
injection

47 .025 (expected .01 to 2.5 x too high)
59. P C1025/10mol

To Page No.

d & Undrst od by m

Dat

Invented by

Date

Edward Bolany

4 | 4 | 9

Recorded by

3-23-55

160

Project No. _____
Book No. _____

TITLE 5% PEI stock

From Page No. _____

Same as P155, 6 except ~~use~~ instead
of using complete Tag ext buffer (P167,3)
just use 50 mM Tris HCl pH 7.5, 1 mM EDTA

A = 50 mM Tris pH 7.5 275 mL
1 mM EDTA

(5526 uA B6C) PEI 50% 50 mL

stir $\geq 50'$

adjust pH to 7.4 with HCl

total A to Vf = 500 mL

To Page 1

Witnessed & Understood by me,

Deborah Boland

Date

4/4/95

Invented by

R. cordey

Date

3-24-95

grow 2L Tf1-106

Exhibit 59
Project No. _____
Appl. No. 09/558,421
Book No. _____

161

Ag N. make 2x LB (lit 40g/L) of LB with base
eg as per P 119, b for O.t.o.k

make 20 ml ON of Tf1-106
in LB + (100 µg/ml Amp, 30 µg/ml Tet
(Mann & uses 15-20 µg/ml
Tet)
10 mg/ml

Ampicillin (Sigma A-9518) 2 g

H₂O 200 ml

filter sterilize

innoculate 10 ml ON / 1L LB

start shaking at 30°C at 7:20 AM

start 7:20 AM A550
12:30 PM 0.567

induce each at 42°C, 15' - by rapidly
bringing up to 42°C in 1' with tap water bath
and then 42°C in water shaker 15'

J

37°C 1 hr in ~~air~~ air shaker
cool in ice water bath

end 1 hr 31°C at 2:05 and 2:35 respectively

OD₅₅₀ final = 0.812 \Rightarrow recovered 5.64g cells

To Page No. _____

I understand by me,

Leanne Polcyn

Date

4/4/95

Invented by

R corded by

Date

3-26-95

3-27-95

Project No. _____

Book No. _____

TITLE _____

buffers for 50g TFI prep

Fr m Page No. _____

follow v tag PRP 91342 • PRP * except for a
2m KCl in

2L

buffer B

1L

buffer
see

1M K phos monobasic ✓ ✗ 34.2

17.1 ml

1M K phos dibasic ✓ ✗ 15.7

7.9 ml

glycerol

✓ ✗ 160

JG

KCl

✓ ✗ 7.46 g
(50 mM)(149.12
(2m))

EDTA 0.5M

✓ ✗ 0.4 ml

0.2 ml

β ME 14.3M

✓ ✗ 700 µl

350 µl

H2O to 2L

1L

Buffer C is 2M/KC
here in order to de
activation point - in
air tag PRP C
700 µm KCl

To Page N

Witnessed & Understood by me,

Deanne Polanyi

Date

4/4/95

Invent ed by

Rec rd d by

Date

3-27-95

AmSO₄ optimization for TfI
(can see P 22, 7 for Tag)

Project No. _____
Book No. _____

Exhibit 61
Appl. No. 09/558,421

163

ag N -

3.64 g TfI cells (P161)

18 ml Tag ext buffer (P167,3)

sonicate

heat treat 75°C, 30 min

PEI

adjust to 200 mM NaCl
Vol = 20 ml so add 1.33 ml NaCl 3M

add 5% PEI (P160) to C_f = 0.4%
stir 15 min (1.7 ml of 5% PEI)

Centrifuge SS 34 15' 15 K
reserved 17 ml sample = Fr I'/PEI

start 11:30 AM
stir AmSO₄ in 15', spin
SS 34 15 K, 15 min

	AmSO ₄
1	Fr I'/PEI
2	2.45 g
3	.493
4	.51
5	.527
6	.527
7	.544
8	.561
9	.561
10	.578

abs
541

25

30

35

40

45

50

55

60

65

70

To Page No. _____

Ised & Understood by me,

researcher's Name

Date

4/4/95

Inv nted by

Recorded by

Date

3-29-95

Project No. _____
Book No. _____

TITLE

B
Polarasay of AmSO₄ supers

rom Page No. _____

assay 2 μ l of $1/100$ dil of each super in 48
 ml Rxn mix (P-120) for 15 min at 74°
 稀释 with 10 μ l EDTA
 spot 401

Bradford

I / PEI 20

AmSO₄ 25%

3.0

3.5

4.0

4.5

5.0

5.5

6.0

6.5

7.0

I / PEI / 70%

20/20

BSA 1 mg/ml

1

2

4

6

8

10

AmSO₄

CPM1

$$\text{u}/\mu\text{l} = 0.64$$

%

0

1

25

30

35

40

45

50

55

60

65

70

75

80

85

90

95

100

100

10706.00

93

11635.00

100

10329.00

90

7609.00

66

803.00

7

465.00

4

514.00

4

258.00

2

313.00

3

230.00

2

Blank

126.00

106668.00

21

13

Date

4/4/95

Invented by

R. Jack

Recorded by

R. Jack

Date

3-30-95

T Page N.

In ssed & Understo d by me,

Date

Invented by

Dat

Dearmer a Boland

4/4/95

R. Jack

R. Jack

3-30-95

BSA

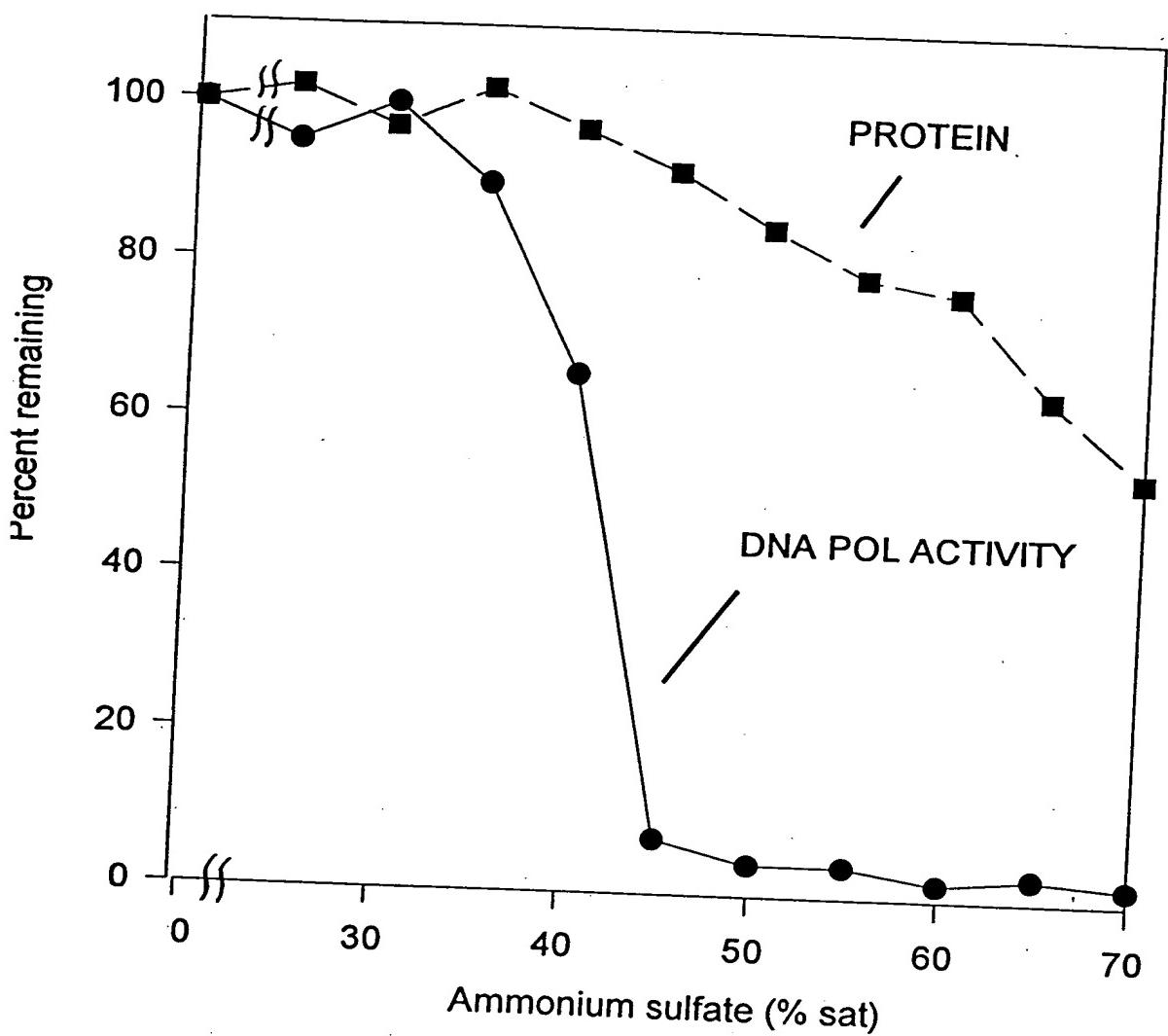
Project N

Bo k N

165

Fig No

Precipitation of Tfl DNA polymerase



To Page No.

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Date

4/4/95

Inv nt d by

Recorded by

Date

Proj ct No. _____
Book No. _____

TITLE port a 180 ml sephadyl Z20

From Page No. _____

in a Pluramain 2.6 XK

1. make slurry of cold sephadyl Z20 - want a
1.5 x vol of pack vol

$$\therefore 1.5 \times 200 \text{ ml slurry} = 300 \text{ ml}$$

2. add 100 ml col buffer (buffer B p 162)
or vol now = 2 x pack vol

and reservoir and gravity flow (set 50 ml/min with effluent tube 2
below bottom of column and 100 mm reservoir) - w/4 col vol/hr

bed volume ended up at 175 ml (2.6cm x 3.5cm)

5 well 25 ml (bed vol) of Blue sephadyl (Ph
L6B) in buffer B (p 162)

since dry swells 4 x 6.25 g

To Page No.

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Deborah Polans

Dat

4/4/95

Invented by

Record d by

Date

4/31/95

Stability unit assay for Tag
same as p121 and 152

Project No. _____
Book No. _____

Exhibit 64
Appl. No. 09/558,421

167

No. _____

note stability study tube #10 (unit assay #29-30)
get 0.01% Dexam or NP40 each added to reactor
by adding 0.5 ml of 1% stock

tube 51-56 =

Cf

Dextran	1.25 mg/ml	1 ml	0.025
	2.5	1	0.05
	5	1	0.1
	10	1	0.2 mg/ml
	10	2	0.4
	10	3	0.8

51 19252.00
52 19303.00
53 18777.00
54 19582.00
55 17015.00
56 17487.00
57 267.00
58 104554.00

→ 65.3 cpm/μml

To Pag. No. _____

ed & Understood by me,

Maria Polley

Date

4/13/95

Invented by

Recorded by

Date

4/4/95

Project No. _____
 Book No. _____ TITLE u/l on P122

From Page No. _____

SAM CPM1

1	13329.00
2	14243.00
3	14542.00
4	14132.00
5	13839.00
6	13367.00
7	14361.00
8	14576.00
9	14684.00
10	11765.00
11	12054.00
12	11446.00
13	13666.00
14	13091.00
15	12913.00
16	10381.00
17	10049.00
18	10787.00
19	16428.00
20	14956.00
21	15556.00
22	15357.00
23	14468.00
24	13489.00
25	14348.00
26	12027.00
27	13354.00
28	9416.00
29	8913.00
30	9177.00
31	13920.00
32	13672.00
33	13373.00
34	14628.00
35	13728.00
36	15178.00
37	14616.00
38	14209.00
39	15366.00
40	14402.00
41	14584.00
42	15003.00
43	12819.00
44	13391.00
45	13180.00
46	16169.00
47	18733.00
48	18552.00
49	16396.00
50	12907.00

u/ml assuming
rTag is .04u/l in 1/125ml ✓ 060
open u/ml P120

.032 .037 .060
 8603

.032 .033 .97

.033 .032 .97

.027 .029 .93

.030 .033 .91

.024 .027 .89

.034 .033 .105

.033 .035 .94

.030 .031 .97

.021 .021 .100

.032 .032 .100

.033 .034 .97

.034 .031 .109

.034 .031 .97

.030 .032 .94

17463 ave (.04u/l)
 by definition

*fn#10, we do not use P120 for
 dat marked as a single point

To Pag No

Witnessed & Understood by me,

Date

Invented by

Dat

Deacona Polans

4/13/95

Rec rd d by

4-4-55

Sample No.	P12 in Other point	1 month	2 months	4 months
.103 TN		84	86%	97
.202 BJ		94	97	98
.202 TX		94	97	106
.016 TN		86	93	93
.020 BJ		85	91	105
.020 TX		89	89	98
103 TN		88	103	104
202 BJ		83	94	91
202 TX		90	97	99
No detergent		95	95	99-
1.1 X		97	100	94
5-X		93	97	100
2x P2GE 0.1%		100	109	(35)
2x Tf 0.02%		94	97	89
2x Vent		95	94	97

To Page No. _____

I & Understood by me,

Alma Polcay

Date

4/13/95

Invented by

Alma Polcay

Dat

4-4-95

Project No. _____

Book No. _____

TITLE _____ Tf1 growth of 4-4-95

70

From Pag No. _____

got ~ 0.6-0.8 g cells from 50ml samples taken at 0 1 2 3 4 hr post induction for 10L per of minimal media (001R) and the same for buffered rich (002R)
plus 50ml at end (~4h post induction) for (002R) plus 114g bulk
Chopped off 2.55g of bulk for 4hr 002R sample

Resuspend cells in Teg ext buffer (P167,3)

add 25 ml ext buffer \Rightarrow 0.9 g cells/ml

sonicate 3 x 10 sec max setting microtip

microfuge 15 min, supernatant = Fr I

90°C 5 min

microfuge 15 min - supernatant = Fr I'

32.2 rpm (2 cm)
pol assay is 2 µl of 1/100 and 1/500 dil
of Fr I'

		pmol u/l	¹⁵ C labeled AUC 1000	Mg/ml	Mg
0150	1	874.00	0.548	1.03	306
	2	440.00			
	3	6172.00			
	4	1538.00	2.6	1.28	2029
	5	5174.00			
	6	1058.00	1.8	1.23	1467
	7	6330.00			
	8	1537.00	2.6	1.27	2050
	9	5734.00			
	10	1206.00	2.1	1.25	1675
	11	1058.00			
	12	324.00	0.58	0.659	314
	13	3961.00			
	14	1227.00	2.1	1.29	1626
	15	4250.00			
	16	1009.00	1.7	1.33	1282
	17	4730.00			
	18	1046.00	1.8	1.21	1493
	19	3435.00			
	20	763.00	1.3	1.27	1018

To Page No.

Witnessed & Understood by me,

Devereux Polas

Date

4/13/95

Inv nted by

Recorded by

Date

4-5-95

114 g Thomas F/c
mini gaukin

Pr j ct No. Exhibit 66
Book N . Appl. No. 09/558,421

171

g N.
cells 9504-02-767-03-002R
(4 hr after induction)

Follow vTag PRP Document # 91342. PRP

114 gram cells

450 ml Tag extract buffer (buffer A)

with fresh β -ME + 5° μ g/ml PMSF

\sim 564 ml (~ 0.2 g cells/ml)

one pass thru mini gaukin 10,000 PSI

heat to 75° C ~~55~~ ($\sim 15'$)

in 40° C water bath

15 min more at 75° C \rightarrow cool in ice slurry

Adjust NaCl to 200 mM

have 550 ml Fr I' (ie after heating)

add 6.43 g NaCl

PEI adjusted to 0.4% by adding

47.8 ml 5% PEI pH 7.4 slowly, then
stir 15 min more

To Pag. No.

Sed & Understood by me,

Deborah Polley

Date

4/13/95

Invented by

J.P.M.

Date

4-7-95

Recorded by

Project No. _____

172

Book No. _____

TITLE _____

From Page No. _____

Spin 30 min in GSA 13,000 RPM

J Ammonium sulfate

Recovered 506.6 ml of Fr I''/PEI

want 47.5% Am(SO₄)₂ saturation

$$= 295.5 \text{ g / L}$$

so add 149.7 g to 506.6 ml Fr I''/P

add slowly, stir 30 min more

centrifuged GS-3, 2500 rpm, 60 min

- Am SO₄ pellet was coming off sides of bottle
 after 60 min spin
 looks like ~~slightly~~ ^{denser} solution

will try 2 hr at 13000 RPM in GSA

27.000 g compared to ~12000 for GS-3

- used smaller bottles (~138 ml / bottle in 4 bottle
 result: pellets won't float)

- collected ppts in filter and rinsed into
 32 ml 1/4 of clear filtrate

- spin 30 min in SS-34 1PK

and spin 1 ml of 32 ml total in microfuge for
 unit assay.

To Pag No. _____

Witnessed & Understood by me,

Deborah Polans

Date

4

13/93

Inv nted by

Record d by

Date

4-7-93

JN

3 N

Try diluting 1:1 the suspended AmSO₄ ppt
+ Tag ext buffer lacking glycerol (ie 50 mM
Tris HCl pH 7.5, 10 mM KCl) plus 47.5%
Saturated AmSO₄. ie, the only effect is
to reduce Cf of glycerol from 8% to 4%
to see if ppt will pellet better.

Result: ppt floats in 4% and also no glycerol!
it does ~~not~~ sink in H₂O

Result:

see p 176 - cells induced only 1 hr don't
have problem of AmSO₄ pellets not sinking
must be too many lipids in cells used here
from 4 hr fermentation time point!

To Pag. No. _____

d & Und rstood by m ,

Date

Invented by

Date

see end of day

4/13/85

Recorded by

7/27/85

74

Project No. _____

Book No. _____

TITLE _____

From Page No. see P154, 3-13-95. Samples have been at room Temp 2^o
 assay same as P121, 152.

0.5 M

Tcaps 200 ml pH 9.3 (at room Temp)
 (243.3 mw) (Sigma T-^{cat#} 5130)

24.33 g + ~140 ml H₂O

2M KOH to pH 9.5

H₂O to 200 ml

tube #1 - 3^o is stability study 1E-15 E in duplicate.
 note tubes 19, 20 (no diluent) gets 0.5 ml of 1% Tween 20/K
 24, 24 in the reactions.
 (ie sample is stability study)

To Page No. _____

I am ssed & Understood by me,

Dorothy A. Polans

Date

4

13 Febr

Invented by

Recorded by

Date

4/11/95

4/11/95

Results of P1/4
Crash TPI same as P

Project N _____

Book No. _____

175

SAM	CPM1	ave	$\frac{a}{1}$	from P1/2 TIME	to of P1/2 values
1	26896.00			1.00	
2	27150.00			1.00	
3	26135.00			1.00	
4	25462.00			1.00	
5	26896.00			1.00	
6	22094.00	22048	.033	.037	89
7	22002.00			1.00	
8	22874.00	22955	.035	.033	106
9	23036.00			1.00	
10	21345.00	22335	.034	.032	106
11	23325.00			1.00	
12	17420.00	17637	.027	.029	93
13	17853.00			1.00	
14	19189.00	19800	.030	.031	91
15	20491.00			1.00	
16	14064.00	14229	.021	.027	78
17	14394.00			1.00	
18	19638.00	20657	.031	.031	94
19	21673.00			1.00	
20	22693.00	20245	.031	.031	89
21	17798.00			1.00	
22	17031.00	18271	.028	.031	90
23	19511.00			1.00	
24	804.00			.022	0
25	710.00			1.00	
26	17770.00	18729	.028	.032	88
27	19687.00			1.00	
28	166725.00			.084	
29	170523.00			1.00	
30	19772.00	18521	.030	.031	97
31	20070.00			1.00	
32	21891.00	19376	.029	.031	85
33	16862.00			1.00	
34	24156.00	22789	.034	.032	106
35	21422.00			1.00	
36	1454.00			1.00	
37	134586.00			1.00	

To Page No. _____

I'd & Underst od by me,

Erica Polk

Date

5/1/95

Invent d by

Peller

Date

4/11/95

Recorded by

176

Project No. _____

Book No. _____

TITLE _____

(Crack Tf1 same as p 171)

From Page No. _____

Fresh cells only grown 1 hr post induction

cells are 9504-10-767-03-003R

grown 4-11-95

resuspend 110 g cells in 440 ml (at room
temp buffer (P167, 3) but no detergent
10,000 ^{PSI} on ^{mini} sartorius, 1 pass
Bring to 75°C in 50°C water (\approx 10 min)
75°C for 15 min more.
cool in ice slurry

Add NaCl to 200 mM Cf

Fr I vol = ~~510~~ 510 ml
so add 5.96 g NaCl

Add PEI (5% stock pH > 4) to Cf = 0.

(used 0.4% last time (P 171) but want to get on
as much DNA as possible)

add 50.4 ml 5% PEI to 510 ml Fr I + N.

\Rightarrow Cf = 0.45% add PEI dropwise and
stir 1.5 min more

spin GSA 13,000 rpm 30'

recovered 49.5 ml supel (= Fr I/1)

To Page N

Witnessed & Understood by me,

Date

Invented by

Date

Deneen Powers

5/1/95

Recorded by

4-13-95

P116 continued

Experiment done on P. 123

Pr j ct N _____ Exhibit 70

Book No. _____ Appl. No. 09/558,421

117

age N

Still Needed 3

cut with Dra I to see if full length lac Z is present
(assuming either Af I or Aa + II recognition sites
had a point mutation generator). Therefore the "40" and "46" bp
miniprep # 54, 58, 64, 73, 87, 98, 103, 108, 113, 123

plus Aa + II, Af I

cut with Sst I to see if R' site in MCS was
a point mutation (or very small deletion)
(see on P(07 at bottom) resulting in the "90's"

miniprep # 3, 29,

Recut with 17 μ l miniprep and load 30 μ l?

^{2.5 μ l water}
trying to resolve the "No results"

miniprep # 20, 39, 71, 74, 75, 76

To Page No.

ed & Understood by me,

me & Polans

Date

2/16/95

Invented by

Recorded by

Date

1-31-95

Project No. _____
Book No. _____

TITLE Sephacyl 200

From Page No. _____

resuspend entire Am 524 pellet in buffer B (P.
added 3 ml to ~1ml pellet.

spin SS34, 13 K RPM, 5 min

add ~200µl buffer B to pellet
respin → 200µl buffer B, more

need to microfuge 15 min to clarify

$V_f = 3.5 \text{ ml}$ (~~~1.9%~~ of 180 ml G100 col)

Load on 180 ml sephacyl 200
elute with 1/2 col vol/hr buffer B (ie 1.5 ml/min)

note pol started coming off
column at 98 ml

98 ml / 180 ml col vol ≈ 54% col vol

To Page N .

I attest & Und rsto d by me,

Devereux Polans

Date

5/1/95

Invented by

Record d by

Date

4-18-95

PAGE 179 OF NOTEBOOK WAS BLANK

Project No. _____
Book No. _____Standard
TITLE TFI unit assay

From Page No. _____

mix used by epinephl same as Tag unit assay (P125)
except only 160 µg DNA/ml instead of 500 µg
in Rxn

A

0.5 M TAPS pH 9.3 15 µl
1M MgCl₂ 6 µl
3M KCl 5 µl
Vf = 2.060 µl

"TFI Rxn mix"

A ✓
229 µl

10 mM TAPS	6.67 µl	✓	2.0
3.7 mg/ml DNA	144.2 µl	✓	160
1.0 ml 10 ³ µCi/ml ³ P dCTP	6 µl		
H ₂ O	<u>275.4</u>	✓	
	<u>3.2</u> µl		

use 40 µl / 150 µl reaction

To Page N

Witnessed & Understood by me,

Deborah Polanyi

Date

5/1/95

Invented by

Recorded by

Dat

4/1/95

ag N TAKETT 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

Rxunin p180 4 μl

1PEI 1/100 dil 2 μl

resuspended 1/1000 dil 2 μl

zonal col fractions 1/100 dil 2 μl

4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

1μl 100 dil

1P09B $V_f = 5 \mu\text{l}$, 74°C , 10 min

2 -

$2 \lambda \frac{1}{100}$
Ans₂₄ super
(2.4 ml m
Ans₅₀₄ in
reorder)

SAM	CPM1	pmol	μl/ml	total units
PEI	1 4110.00	163	2.45	1.21×10^6 units in 455 ml]
yield	2 6087.00	362.9		1.28×10^6 in 3.5 ml Ans ₂₄ resuspended
4	3 308.00			
5	4 356.00			
6	5 678.00			
7	6 3373.00			
Y	7 8181.00			
9	8 11817.00			
10	9 9111.00			
11	10 8925.00			
12	11 5943.00			
13	12 2583.00			
14	13 1385.00			
15	14 773.00			
16	15 351.00			
17	16 299.00			
18	17 304.00			
19	18 245.00			
20	19 407.00			
F1	20 2651.00	105	1.5 μl (expected only 1μl in stock from supplier)	
6-B	21 358.00			
super	22 818.00			
mix	23 60259.00			
		37.7 cpm/μmol		

7-12

for fr~~7-11~~ = average of ~8000 cpm for 1μl

$\Rightarrow 47.7 \mu\text{l}/\mu\text{l} \Rightarrow [859,000 \text{ total units}/1\mu\text{l}]$

or ~72% recovery from FrI/PEI

To Page No. _____

I have read & Understood by me,

Barbara Polansky

Date

5/1/95

Invented by

R. C. Reddy

Date

4-17-97

182

Project No. _____
Book No. _____

TITLE

Blue sepharose

From Page No.

load pooled sephadex 200 fractions #7-12 (18 ml V to
on 20 ml Blue at 0.35 ml/min (~1 col vol)
wash 5 col vol o/N at 0.16 ml/min buffer
gradient is 40 col vol (use buffer B-C, p162)
at 3 col vol/hr = 1ml/min, 6 ml fraction

Buffer B

in Tris pH 7.5
0.5 M EDTA
Glycerol
β Me
KCl

* D

200 ml ✓✓
1.6 ml ✓✓
640 ml ✓✓
2.8 ml ✓✓
29.8g

E

25 ml 12.5 ✓✓
0.2 ml 0.1 ✓✓
20 ml 40 ml ✓✓
350.2 ml .175 ✓✓
14.9g ✓✓
74.5g ✓✓
500 ml

PL (50 mM KCl)

2 M KCl

(note buffer D is
7.5 M KCl in Tag Prop 91342
but only 50 mM here)

enter "2" to get to bank 2

then 5

HOD 5 BANK 2

.00	CONC	LB	A	0
.00	CONC	LB	0	0
.00	ML/MIN		1	
.00	SP. DENSITY			

400 =
400 =

still 1

a

To Page N



Assisted & Understood by me,

James A. Polley

Date

5/1/95

Invented by

Recorded by

Date

4/15/95

pool Blue fractions 24-32 based on UV profile
 $V_f = 54 \text{ ml}$

Dialysis against 5L buffer D (P182) o/N
recovered ~ 60 ml

Conductivity 10 μl in 1ml H₂O

Buffer D
in vol effluent
to equilibrium o/N

$$\frac{101 \mu\text{s}}{98} = 10.1 \text{ mS}$$

$$9.8 \text{ mS}$$

Dialysate 99 9.9 mS

(can see P 41 where results are similar)
for Tog

To Page No. _____

ed & Understood by me,

enrich Polkay

Date

5/1/95

Invented by

Recorded by

Date

4-20-95

PAGE 184 OF NOTEBOOK WAS BLANK

Heparin AF (20ml col) Pr j ct No. _____

185

le N - Equilibrat 0/N with buffer D, P182 (50mM KCl) → see P183 for conductivity of col effluent
 (load) ≈ 60 ml dilysate (P183) at 0.67 ml/min
 = 2 col vol / hr (as done on P11 for rTag)
 (1 min/min)

wash 1 col vol 0.67 ml/min

for gradient want to make it fairly flat for first try
 of TFI on Heparin.

Gradient:

50 - 700 mM KCl (= 0-35% pump B since
 E is 2 mM KCl)

20 col vol = 400 ml, 4 ml/hr (so 100 portions total)
 run at 2 col vol/hr
 need 10 hours for whole gradient

rTag comes off Heparin at 400 mM KCl (see P 46)
 might see TFI at 6 hr point in a late afternoon
 if TFI same as Tag

(loading done at 10:25 AM, wash 30 min (= 1 col vol)
 gradient start at 11 AM)

IOD 5 BANK 1

```
.00 CONC .4B
.00 CONC .4B
.00 ML/MIN
.00 PORT. SET
.00 PORT. SET
.00 VALUE.POS
.00 VALUE.POS
.00 CONC .1S
.00 ML/MIN
```

To Pag N _____

ed & Und rstood by m ,

meena Polkay

Date

5/1/95

Invented by

R cord d by

Date

4/20/95

Exhibit 74
Appl. No. 09/558,421

Project N _____
Book No. _____

187

SDS-PAGE Heparin fractions

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

32.5 ~~5~~¹⁰ / ~~present~~

Cocaudinella agathae

4 Jul as corrected

Paul Foster

To Page N

Witnessed & Understood by m ,

Data

Inv nted by

Dat

Dear Mr. Phipps

5/1685

R c rd d by

4-21-97

age N _____

Wintō

~~recovery~~

1'/PEI

1,210,000

100%

monium sulfate

1,280,000

100

phacryl 200

259,000

71

re sepharose

756,000

62

alyris

751,000

62

zarin AF

666,000

55

To Page No. _____

d & Understood by me,

Dat

J

Invented by

R corded by

Date

4-21-15

190

Project No. _____
Book No. _____

TITLE

From Page No.

Tet stock

grow λ phage sp6 plasmid
in host lacking TFI sp6 plasmid
containing Tet resistance gene
plasmid with λ promoter and
sp6 gene

LB 50 ml

Tet 30 μ g/ml

add 50 mg cells \rightarrow 30°C shaking 0/1

got no growth 0/N 1 4-24

→ no it just grew
slow - had cells 36 h

Repeat with M Lysogen Tet ad and

at only 15 μ g/ml

0/0 7/7 (including stock used above that &
after 36 h) grow 0/N at 30°C

should dissolve "crystallizing" TC in E

made fresh ~~Tet~~ TC stock

139 mg Sigma No T3258 Tetracycline

a little water

(it still doesn't go into solution)

100% EtOH (good stuff from Cor Nov)

up to 27 ml

= 5 mg/ml store in foil, -20°C

innoculate 1 ml of 0/N #5 into 50 ml
E. coli grown + ~~50~~ μ g/ml of fresh TC stock
shake at 30°C 30 μ g/ml

start T. 30 stop 3 pm

to add 0.88 ml Tag et buffer (P167,3) (for 0.2 mg/ml)

Witnessed & Understood by me,

Deeann A Polley

Date

5/1 (95)

Invented by

Recorded by

To Page N

Date

4-23-95

Regeneration of columns

Project No. _____
Book No. _____

Exhibit 76
Appl. No. 09/558,421

191

Re. No. _____

Blue sepharose

2 col vol 6M Guanidinium HCl
5 col vol H₂O (immediately)
2 col vol 20% EtOH for storage

Heparin Af

2 col vol 4M urea
2 col vol H₂O
2 col vol 20% EtOH

(O. 3 = 0.5 M NaOH recommended)

sephadex S200

1/2 - 1 col vol 0.4M NaOH

contact with col = $\geq 1 \text{ hr} \leq 2 \text{ hr}$.

H₂O 2 col vol

20% EtOH for storage

run 0.4M NaOH at 2 ml/min
for 45 min (= 1/2 col vol)

(start 10:20 am) H₂O for 3 hr at 2 ml/min
= 2 col vol and NaOH only in
contact with column for
45 min + 90 min

20% EtOH 3 hr 0.2 ml/min 0/N

To Page No. _____

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Date

Invented by

Date

seen and signed

5/1/95

Recorded by

4.24.95

Project No. _____

Book No. _____
MW

TITLE _____

SDS gel for TfI prep

192

From Pag No. _____

↓

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
MW ↓

DH10BRK2

Fr I P190

Fr I' (75°C, 30) P190

3λ

30λ

TfI Fr I 2.45μl (0.02%)

3λ

Fr I' 2.45μl (P181)

30λ

Am SV4 resuspended

0.5

3.62μl

5200μl 50μl

3.5

Blue pool Fr 24-32

12.5

14μl (P178)

Heparin Fr #

39 3μl

5

40 9.9

5

41 21.9

5

42 40.5

5

43 41.3

5

44 43.7

5

45 55.6

5

46 12.3

5

47 4.5

5

TfI apiculate 1 μl

30

ext TF31010A-502

2X sample buffer

30

H2O

.27 - .27 - .30 27.17.525

→

→

load 15μl MW standards

(CTI cat 10064-01)

run at ~29 mA

started 9:15 AM

T Page No. _____

I am ssed & Understood by me,

Deborah Polley

Date

5/1/95

Invented by

R c rd d by

Date

4-25-91

47 cm Heparin AF column

Pr j ct N _____

Exhibit 78

Bo k N _____

Appl. No. 09/558,421

1

je No.

poored on 1.5 cm x 47 cm (80 ml) column
to try to separate the 2 peaks on P186-187, 9
flow rate is 0.204 ml/min by gravity.

gradient will be 50 mM - 400 mM

and 10 col vol = ~~100 ml~~ 1600 ml

so gradient 1/2 as steep as P185, 9 : 20 ml col

pool for 40-43 (14.7 ml total)
of Heparin (see P189-192, 9)

Dialyze ON against 1 L buffer ▷

(frns are ~ 300 mM KCl

so expect ~ 4.2 mM + 50 mM in buffer)

start gradient ~ 9:30 AM

gradient is

1600 ml (20 col vol)

50 mM - 400 mM KCl (was 50 mM - 700 mM on)

13.3

2 ml in 5 ml/min, so after hr for gradient

4.5 min / frn = 9 ml / frn (200 frns total)

note 1.5 ml/min gave only 0.2 mPa (column
is definitely running with backpressure) but
2 ml / min still only ~ 0.2 mPa so will
use 2 ml / min \Rightarrow 1.5 col vol / hr
used 2 col vol / hr for 20 ml col P185, 9

To Page No. _____

Ied & Understo d by me,

Robert D. Sorenson

Date

5/1/95

Invented by

Record d by

Date 26-95

4.27.95

From Page No. _____

expect protein to start coming off at $\approx 65\%$ of the gradient is $\approx 1040 \text{ ml} = 8.7 \text{ hrs}$.
 or $\approx 6:30 \text{ pm}$

since prot started coming off at $13\% \text{ D} + 50 \text{ mM}$
 $\approx 410 \text{ mM}$

Comparison of 80 ml and 20 ml columns

col vol	20ml	80ml
col height	11 cm	47 cm
gradient vol	20 wl vol	20 col vol
gradient stop	<u>35 mM KCl</u> col vol	<u>20 mM KCl</u> wl vol
flow rate	2 wl vol/hr	1.5 wl vol/hr.

Therefore the new col is $4\times$ longer, has $0.75\times$ flatter gradient and is $0.75\times$ slower flow rate
 so hope to get better separation of 2 peaks see
 on p 186-187, 9

THOD 5 BANK 2

1.00	CONC %B	C
1.00	CONC %B	C
.00	ML/MIN	2.
.00	PORT. SET	3
.00	PORT:SET	6
.00	VALUE.FOS	1
.00	VALUE.FOS	2
1.0	CONC %B	20
1.0	ML/MIN	3.

Witness d & Und rsto d by me,

Devereux Polkay

Date

5/1/95

Invented by

R corded by

Date

4-27-95